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16. ABSTRACT

Fathead minnows and goldfish were compared for their suitability as a standard bioassay fish. Acute bioassays of four potential reference toxicants, sodium chloride, pentachlorophenol, hexavalent chromium, and Guthion^{R}, were conducted with both species, and results were reported as toxicity curves as well as LC50's at various times. Both species showed the same variability of bioassay results. Since goldfish could not complete a life cycle in 1 year under laboratory conditions, fathead minnows were recommended as a standard species on the basis of their smaller size and their utility in complete life cycle tests.

Bioassays of pentachlorophenol were conducted with both species to determine the effect of testing different sized fish of the same age or testing different aged fish. Size selection of fish within the ranges tested is unnecessary since differences in LC50's were small. Since age of fathead minnows did not affect the LC50's after 24 hours, use of younger fish would allow smaller bioassay chambers or more fish per chamber.

On the basis of seven criteria, sodium chloride was superior for use as a reference toxicant with pentachlorophenol a close second choice.

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July 1976

STANDARD TEST FISH DEVELOPMENT

PART I

Fathead Minnows (*Pimephales promelas*) and Goldfish (*Carassius auratus*) as Standard Fish in Bioassays and Their Reaction To Potential Reference Toxicants

by

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ABSTRACT

Fathead minnows and goldfish were compared for variability of LC50's with four toxicants as a means of determining their suitability as a standard bioassay fish. Acute bioassays with sodium chloride, pentachlorophenol, hexavalent chromium and Guthion® were conducted with both species; and results were reported as toxicity curves as well as LC50's at various times. Both species showed similar variability of bioassay results.

Bioassays of pentachlorophenol were conducted with both species to determine the effect of testing different sized fish of the same age or testing different aged fish. Size selection of fish within the ranges tested is unnecessary since differences in LC50's were small. Since age of fathead minnows did not affect the LC50's after 24 hours, use of younger fish would allow smaller bioassay chambers or more fish per chamber.

Since goldfish could not be induced to complete a life cycle in the laboratory in less than one year, fathead minnows were superior as a standard species because of their smaller size and their utility in complete life cycle tests.

On the basis of seven criteria, sodium chloride was superior for use as a reference toxicant with pentachlorophenol a close second choice.

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CONTENTS

		Page
Abstract		iii
List of Figures		vi
List of Tables		vii
Acknowledgements		x
Sections		
I	Conculusions	1
II	Recommendations	2
III	Introduction	3
	Standard Fish Studies	3
	Reference Toxicant Studies	5
	Determination of Suitable Size and Age	6
IV	Materials and Methods	8
	Acute Tests	8
	Goldfish Culture Study	27
V	Results	33
	Acute Tests	33
	Goldfish Culture Study	56
VI	Discussion	67
	Selection of a Standard Species	67
	Selection of a Reference Toxicant	69
VII	References	71
VIII	Publications	74
IX	Glossary	75

FIGURES

No.		Page
1	Spatial Arrangement of Test Chambers Drawn to Scale in Goldfish Culture Study.	28
2	Toxicity Curves of Sodium Chloride, Pentachlorophenol, Guthion®, and Hexavalent Chromium for Goldfish and Fathead Minnows.	34
3	Toxicity Curves of Hexavalent Chromium for Goldfish and Fathead Minnows.	41
4	Relationship of Reciprocal of LC50 Versus Reciprocal of Time for Goldfish of Different Sizes and Fathead Minnows of Different Sizes and Different Ages.	55

TABLES

No.		Page
1	Analysis of Well Water	9
2	Acute Toxicity and Mean Test Conditions in Bioassays to Determine Variability in Response of Fathead Minnows and Goldfish to Sodium Chloride	12
3	Acute Toxicity and Mean Test Conditions in Bioassays to Determine Variability in Response of Fathead Minnows and Goldfish to Pentachlorophenol	14
4	Acute Toxicity and Mean Test Conditions in Bioassays to Determine Variability in Response of Fathead Minnows and Goldfish to Hexavalent Chromium	16
5	Acute Toxicity and Mean Test Conditions in Bioassays to Determine Variability in Response of Fathead Minnows and Goldfish to Guthion®	18
6	Test Conditions in Bioassays with Large and Small Fathead Minnows of Constant Age	20
7	Test conditions in Bioassays with Fathead Minnows of Various Ages	21
8	Test Conditions in Bioassays with Large and Small Goldfish from a Constant Stock	22
9	Diet of Two Groups of Goldfish During Different Periods of the Experiment	32

No.		Page
10	Means and 95% Confidence Intervals of LC50's of Four Toxicants and Two Fish Species at Various Times	36
11	Means and 95% Confidence Intervals of LC50's of Hexavalent Chromium from Results of the Second Experimental Procedure	42
12	Mean LC50's with Coefficient of Variability of Four Toxicants at Various Times	43
13	Occurrence of Significant Differences in Multiway Analyses of Variance with Four Toxicants	46
14	Comparison of LC50's from Unhealthy Goldfish Stock with Mean LC50's from All Tests with "Normal" Goldfish	49
15	Comparison of Four Toxicants with Regard to Their Use as a Reference Toxicant	51
16	Mean and Standard Deviation of Weight and Length of Fish in All Bioassays	52
17	Regression Equations and Coefficient of Determination (r^2) for the Relationship Between LC50 and Time in Three Groups of Experiments	54
18	Means and Standard Deviations of LC50's with Pentachlorophenol for Different Groups of Fish at Various Times	56
19	Means and Standard Deviations of Conditions in Goldfish Culture Study	58
20	Weight of Individual Fish in All Tanks at Termination after 393 Days	60

No.		Page
21	Mean Weights of Fish from All Individual Tanks at Approximately Monthly Intervals	62
22	Standard Lengths of Individual Fish in All Tanks at Termination after 393 Days	64
23	Sex and State of Gonadal Maturity of Individual Fish in All Tanks at Termination after 393 Days	66

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SECTION I

CONCLUSIONS

The results described in the following report permit certain conclusions concerning the selection of a standard bioassay fish and choice of a reference toxicant.

1. Fathead minnows and goldfish are similar in sensitivity to sodium chloride, pentachlorophenol, hexavalent chromium, and Guthion® in acute bioassays.

2. Variability of LC50's with the four toxicants was similar for both species and was more dependent on the toxicant than the species.

3. Since fathead minnows can complete a life cycle in less than one year and the culture study with goldfish was unsuccessful in achieving this result, fathead minnows will presently meet this criterion for a standard species.

4. Neither size of goldfish nor size and age of fathead minnows affected LC50's of pentachlorophenol at times longer than 24 hours.

5. Sodium chloride best met some of the desired characteristics of a reference toxicant with pentachlorophenol a close second.

SECTION II

RECOMMENDATIONS

1. Fathead minnows are recommended for use as a standard bioassay fish.

2. It is further recommended that the appropriate federal agency promote the development of a private or public hatchery for this species to provide uniform stock.

3. It is recommended that approximately 4 to 7 week-old fathead minnows be used in standard bioassays.

4. Goldfish from a single gene pool are recommended as a standard fish until a source of fathead minnows becomes available.

5. Sodium chloride or pentachlorophenol are recommended for use as a reference toxicant.

6. Further studies are recommended to determine which reference toxicant is more suitable for detection of abnormal fish.

SECTION III

INTRODUCTION

STANDARD FISH STUDIES

Many authors have mentioned the desirability of a standard fish species for use in all types of fisheries research (Marking, 1966; Lennon, 1967; Cairns, 1969; Sprague, 1970). In an extensive discussion on the use of selected strains of fish as bioassay animals, Lennon (1967) stated that the need for standard strains of fish was "immediate and extremely important in view of the expansion of fish bioassays in pharmacology, pollution control, pesticide evaluation, fish culture, and fish control." This need was based on the requirement for reproducibility of test results. Since resistance to a toxicant may vary with species, strain, previous history, age, size, health, and handling procedures, the first step in achieving reproducibility of results within or between laboratories would be development of standard bioassay procedures including standard water and a standard bioassay fish.

Recently various government agencies have been attempting to define standard species and tesing procedures for use by industries to monitor their effluents. The purpose of developing these procedures is to identify and isolate potentially detrimental effluents through bioassay and to determine the relative toxicity of effluents. Since many industrial effluents are complex mixtures whose toxic components are unknown or difficult to isolate, the biological monitor serves as a more rapid and probably more realistic identification tool for potential hazards than a chemical analysis. Industrial personnel who would conduct these tests

are usually not trained fisheries biologists, therefore standard testing procedures and fish strains are necessary to insure valid comparisons of the potential hazard of effluents.

The Ohio River Valley Water Sanitation Commission has developed a standard 24-hour static bioassay using a prescribed strain of goldfish for monitoring industrial effluents (ORSANCO, 1974). MacLeod (1972) has proposed a similar program using rainbow trout to establish uniform enforcement regulations throughout Canada. The Water Resources Control Board of California is also attempting to designate strains of one or more fish species for use in a statewide industrial monitoring program.

The more important criteria for selection of any strain used as a standard fish for bioassay are: 1) relatively constant response to a broad range of toxicants when tested under similar conditions; 2) available in large quantities with close quality control; 3) easily handled for bioassay purposes; 4) easily transported; 5) continuous availability of the desired size; and 6) capable of successful completion of a life cycle in 1 year or less under laboratory conditions.

One objective of the present study was primarily related to the first criterion. If a standard strain is used to make comparisons of the toxicity of industrial effluents, there must be some indication that the strain does not show high variability in response when tested under standardized procedures at different times or locations. To assess variability, acute bioassays were conducted over a 2-year period with two fish species frequently suggested as potential standards and four toxicants with different modes of action, sodium chloride, pentachlorophenol, hexavalent chromium, and Guthion®.

Goldfish, Carassius auratus (L.), were chosen because of their availability in large quantities from a single commercial source, Ozark

Fisheries, Inc., Stoutland, Missouri, and because of their proposed use in a standard bioassay procedure for industries in the Ohio River Valley (ORSANCO, 1974). The fathead minnow, Pimephales promelas Rafinesque, was selected because of successful use in many bioassays (Martin, 1973). A strain of fathead minnows from the National Water Quality Laboratory at Duluth, Minnesota has been available for research purposes and a small-scale hatchery design was developed by personnel at that laboratory (U.S. Environmental Protection Agency, 1971). If the present study indicated that the fathead minnow was more suitable, the technology for development of a commercial source would be available.

A second objective of the present study related to the last criterion listed. Was the goldfish capable of completing a life cycle within 1 year under laboratory conditions? Previous attempts to rear goldfish from egg through spawning adult under chronic bioassay conditions in our laboratory were not successful, although fish that had spent at least the first summer of their life in outdoor ponds readily spawned in our laboratory when mature. Hervey and Hem (1968) reported that field-reared fish spawned as early as 9 months, and pond-reared fish spawned in our laboratory at approximately that age. An experiment was therefore conducted to examine the effect of temperature, food, and crowding on the ability of goldfish to mature in the laboratory. These three variables were suspected as causes of previous failures.

REFERENCE TOXICANT STUDIES

Marking (1966) and Davis and Hoos (1975) have discussed the need for a reference toxicant to determine the condition of fish at the time of testing. In theory a reference toxicant bioassay under standardized test conditions would be conducted just prior to or simultaneously with a bioassay of the toxicant or effluent under consideration. If results from the reference toxicant bioassay deviated considerably from previously accumulated data on this toxicant, the fish stock would be considered abnormal and results of any bioassays with it would be ques-

tionable. This procedure would be useful both to the industry or laboratory conducting the test and to regulatory agencies for determining the validity of test results. Davis and Hoos (1975) conducted a field test of reference toxicants including pentachlorophenol where the materials were bioassayed by different laboratories. This study indicated differences between laboratories but not within-laboratory variability, the basis upon which results would in part be judged. Furthermore, tests were conducted on rainbow trout from a variety of sources rather than a single stock.

As with the standard fish strain, a reference toxicant would have to meet certain criteria to be useful. The more important criteria are: 1) minimum variability in response of normal fish; 2) rapid detection of abnormal fish by a deviant response; 3) rapid lethal action; 4) simple analytical technique; 5) usable in static and flow-through bioassays; and 6) general ease of laboratory handling. Three of the four toxicants used for determination of species variability were selected for their potential as reference toxicants as well as their different modes of toxic action. These potential reference toxicants were sodium chloride, pentachlorophenol, and hexavalent chromium. A third objective of the present study was to determine how well the tested materials met the criteria for a reference toxicant.

DETERMINATION OF SUITABLE SIZE AND AGE

Different shipments of goldfish from Ozark Fisheries varied considerably in age and weight and since weight of these fish is generally not correlated with age, conditions in the rearing ponds are probably responsible for size differentials. With fathead minnows age of fish for testing can be precisely controlled to 1-week age groups, but conditions during rearing result in considerable variability in size of these fish. Since the age of the goldfish cannot be controlled, it is desirable to know what effect size would have when fish are tested with one of the potential reference toxicants. Variation in a reference toxicant bioassay

attributable to size might require rejection of large or small stocks of fish. In using fathead minnows of constant age, different sized fish might also contribute to variability. Since various aged fathead minnows could be selected by use of the culture system (U.S. Environmental Protection Agency, 1971), variation in test results might require use of a constant age in all tests or lack of variation due to age might permit testing fish of a variety of ages or very young fish. Either of these latter alternatives would allow for greater culture unit production or smaller culture units if younger fish are used.

A final objective of the present study was to determine the difference in sensitivity to one of the potential reference toxicants, pentachlorophenol, of different sized fathead minnows and goldfish of a constant age or stock and to determine the difference in sensitivity of different aged fathead minnows.

SECTION IV

MATERIALS AND METHODS

ACUTE TESTS

Apparatus and Water

All bioassays of hexavalent chromium and pentachlorophenol were conducted in the same two flow-through proportional diluters constructed of glass, G.E. Silicone Seal, and Koroseal plastic tubing (Mount and Brungs, 1967). Each diluter dispensed seven toxicant concentrations with a dilution factor of 60% and a control. A series of flow splitters permitted duplication of each toxicant concentration and control so that four separate tests could be conducted simultaneously. The test chambers were constructed of glass and G.E. Silicone Seal, measured 50 x 25 x 20 cm, and contained 20 liters. With a flow rate of 105 ml/min, 90% replacement of water occurred in approximately 7 hours (Sprague 1969) and 7.6 test chamber volumes were added each 24 hours. The pH was controlled by dispensing sulfuric acid into the head tank with a chemical metering apparatus (Mount and Brungs, 1967), and the temperature was controlled by a hot water heat-exchange system in the head tank. Hexavalent chromium was reagent grade potassium dichromate and pentachlorophenol was technical grade sodium pentachlorophenate. The pentachlorophenol was analyzed for dioxins, a frequent contaminant, and none were present.

All flow-through tests were made with water from a deep well, pumped to the apparatus through polyvinyl choride pipe after iron removal (Table 1).

Table 1. ANALYSIS OF WELL WATER[a]

(milligrams/liter)

Item	Concentration
Total hardness as $CaCO_3$	220
Calcium as $CaCO_3$	140
Iron	0.02
Chloride	<1
Sulfate	<5
Sulfide	0.0
Fluoride	0.22
Total phosphates	0.03
Sodium	6
Potassium	2
Copper	0.0004
Manganese	0.0287
Zinc	0.0044
Cobalt, nickel	<0.0005
Cadmium, mercury	<0.0001
Ammonia, nitrogen	0.20
Organic nitrogen	0.20

[a] Water taken from well head before aeration and heating; pH 7.5.

Inability to dissolve adequate concentrations of sodium chloride and Guthion® in the proportional diluters required the use of a renewal bioassay with these materials. The same number and size test chambers were used as for the toxicants tested in flow-through apparatus. Each test chamber contained a 50-watt aquarium heater and was aerated continuously.

Preliminary tests indicated that high pH levels in the aerated laboratory well water resulted in sublethal accumulations of unionized ammonia in static tests. In order to maintain a lower pH and keep the ammonia in a non-toxic ionized form, a soft water was used in these tests. This water was prepared by diluting laboratory well water with 80% deionized water and buffering with 272 mg/liter mono-basic potassium phosphate and 25 mg/liter sodium hydroxide. A fresh preparation of this water was added to each chamber at the start of the acclimation period and on introduction of toxicant, and 88% of the water and toxicant was changed every 2 or 3 days during the test. Chemical analysis indicated that there was no reduction of either sodium chloride or Guthion® under test conditions for up to 4 days. Reagent grade sodium chloride crystals were dissolved directly into each test chamber, and 93% technical grade Guthion® in an acetone carrier was pipetted directly. One control chamber for each species received a volume of acetone equivalent to that of the highest Guthion® concentration (1750 ppm) and no more than 10% mortality was ever observed in this chamber. This 10% loss occurred in the acetone control in two bioassays and also in the control without acetone in two tests.

Fish

Fathead minnows were reared in a laboratory hatchery similar to that designed at the National Water Quality Laboratory (U.S. Environmental Protection Agency, 1971). The original stock of juvenile fish was obtained from the National Water Quality Laboratory at Duluth, and at approximately 8- to 10-month intervals additional spawning stock from Duluth was mixed with the hatchery-reared spawners to prevent develop-

ment of a genetically divergent strain. Stocks of fish could be selected for testing from within 1-week age groups. The fathead minnows tested for the determination of bioassay variability were 11 weeks old at the start of each test, and mean weights ranged from 0.12 to 0.38 g (Tables 2, 3, 4, 5). In studies on the effect of size and age, various ages and weights were used depending on the objective of each test (Tables 6 and 7).

Goldfish were obtained from Ozark Fisheries, Inc., Stoutland, Missouri. Growth of these fish is sometimes intentionally retarded by the supplier to maintain a stock of the desired size. Upon arrival all stocks were treated twice on successive days with 1 mg/liter potassium permanganate for 1/2 hour to remove Gyrodactylus. On the third day they were treated with 25 mg/liter tetracycline. After treatment fish were held for at least 10 days at 21 C in flowing water prior to testing. In tests of bioassay variability goldfish varied in age from 5 months to 1-1/2 years, and mean weights ranged from 1.37 to 2.70 g (Tables 2, 3, 4, 5). In tests for determination of the effect of size, fish were approximately 6 months old and size varied depending on the objective of the test (Table 8).

Bioassay Procedure

Determination of Variability--Eight series of tests were conducted in duplicate for a total of 16 bioassays per species per toxicant (Tables 2, 3, 4, 5). From 68 to 72 hours prior to the introduction of toxicant fish were sorted in a stratified random manner into the two sets of test chambers, with 10 fish in each. After acclimation to the test chamber, test water, and temperature, the toxicant was immediately brought to the desired concentration by pipetting from a stock solution for Guthion®, sodium pentachlorophenate, and potassium dichromate, and by direct dissolution of sodium chloride. In a series of eight tests an error in calculation resulted in about 35% of the desired hexavalent chromium concentration initially with desired concentrations not attained for 3 to 4 hours. In three additional tests with each species

Table 2. ACUTE TOXICITY AND MEAN TEST CONDITIONS IN BIOASSAYS TO DETERMINE VARIABILITY IN RESPONSE OF FATHEAD MINNOWS AND GOLDFISH TO SODIUM CHLORIDE

Test	Temperature, C	pH, meter reading	O_2, mg/l	Weight, g	Length,[a/] mm	LC50, mg/l 24 hr	48 hr	96 hr	Threshold	(days)
					Fathead minnows					
F1U	25.0	7.16	-	0.24	27.2	7100	7050	7050	7050	(4)
F1L	24.7	7.12	-	0.26	27.6	7200	7100	7100	7100	(4)
F2U	25.2	7.24	7.22	0.21	24.2	7400	7400	7400	7400	(4)
F2L	25.0	7.26	7.48	0.26	25.7	7700	7650	7650	7650	(4)
F3U	24.7	7.20	7.13	0.20	23.7	7750	7400	7400	7400	(4)
F3L	25.0	7.17	7.37	0.19	22.8	7500	7300	7200	7200	(5)
F4U	25.1	7.05	6.09	0.25	27.2	9000	8300	8300	8300	(4)
F4L	25.2	7.06	6.14	0.26	27.0	8300	8200	8200	8200	(4)
F5U	25.0	7.33	6.98	0.26	27.1	8150	7800	7800	7800	(4)
F5L	24.8	7.28	6.85	0.26	27.7	--	7650	7650	7650	(4)
F6U	25.0	7.20	7.50	0.24	26.4	7750	7550	7450	7450	(5)
F6L	24.8	7.19	7.53	0.24	27.0	8100	7950	7950	7950	(4)
F7U	24.8	7.33	7.11	0.35	26.9	8800	8700	8400	8400	(5)
F7L	25.0	7.31	7.08	0.32	26.1	8800	8300	8150	8150	(5)
F8U	25.2	7.41	7.12	0.30	27.0	7500	7200	7200	7200	(4)
F8L	25.0	7.41	7.05	0.32	27.4	7600	7500	7500	7500	(4)

Table 2 (continued). ACUTE TOXICITY AND MEAN TEST CONDITIONS IN BIOASSAYS TO DETERMINE VARIABILITY IN RESPONSE OF FATHEAD MINNOWS AND GOLDFISH TO SODIUM CHLORIDE

Test	Temperature, C	pH, meter reading	O_2, mg/l	Weight, g	Length,[a] mm	LC50, mg/l 24 hr	48 hr	96 hr	Threshold	(days)
					Goldfish					
G1U	25.1	7.13	6.93	2.53	35.2	9900	7550	7000	7000	(6)
G1L	25.3	7.11	6.69	2.44	34.4	9750	7350	6950	6950	(6)
G2U	24.7	6.95	6.80	1.79	30.0	9800	7200	7050	7050	(5)
G2L	24.7	7.05	6.38	1.92	31.5	8350	6800	6800	6800	(4)
G3U	24.8	7.20	7.22	1.57	33.0	9850	7950	7600	7600	(5)
G3L	24.9	7.18	7.06	1.65	33.2	10000	7400	7200	7200	(5)
G4U	25.0	6.98	6.38	2.39	33.1	11050	8200	7900	7900	(6)
G4L	24.4	7.01	6.68	2.34	32.8	11050	8400	8050	8050	(6)
G5U	24.7	7.04	7.02	1.53	32.1	10100	7900	7200	7200	(5)
G5L	24.9	7.03	7.08	1.56	31.9	10200	7400	7050	7050	(5)
G6U	25.2	7.16	6.18	1.69	33.8	10450	8800	7950	7750	(7)
G6L	24.9	7.19	6.55	1.65	33.7	10400	8500	7950	7950	(6)
G7U	24.9	7.24	7.31	2.17	37.8	9980	7900	7650	7550	(7)
G7L	25.2	7.19	7.17	2.18	37.8	9600	7850	7300	7300	(6)
G8U	25.0	7.13	7.18	2.77	38.0	8800	7150	7000	7000	(5)
G8L	24.7	7.10	7.27	2.83	38.7	9950	6950	6800	6800	(6)

[a] Fatheads - total length; goldfish - standard length.

Table 3. ACUTE TOXICITY AND MEAN TEST CONDITIONS IN BIOASSAYS TO DETERMINE VARIABILITY IN RESPONSE OF FATHEAD MINNOWS AND GOLDFISH TO PENTACHLOROPHENOL

Test	Temperature, C	pH meter reading	O_2, mg/l	Weight, g	Length,[a/] mm	LC50, mg/l 24 hr	48 hr	96 hr	Threshold	(days)
					Fathead Minnows					
F1U	24.6	7.82	6.52	0.31	28.7	.20	.20	.20	.20	(4)
F1L	23.7	7.83	6.67	0.33	28.8	.18	.18	.18	.18	(4)
F2U	24.9	7.72	6.37	0.19	23.4	.22	.22	.22	.22	(4)
F2L	24.3	7.72	6.61	0.20	23.9	.18	.18	.18	.18	(4)
F3U	25.9	7.69	7.02	0.21	24.1	.20	.20	.19	.19	(5)
F3L	24.7	7.68	7.18	0.18	23.5	.21	.21	.21	.21	(5)
F4U	25.8	7.86	6.44	0.30	28.6	.24	.24	.22	.22	(5)
F4L	25.2	7.78	6.61	0.30	28.4	.19	.19	.18	.18	(5)
F5U	25.8	7.59	6.27	0.22	25.5	.19	.19	.19	.19	(4)
F5L	25.1	7.62	6.39	0.23	25.5	.19	.19	.19	.19	(4)
F6U	25.3	7.65	6.22	0.22	26.0	.28	.27	.24	.24	(6)
F6L	24.2	7.65	6.48	0.24	27.0	.28	.22	.20	.20	(5)
F7U	24.6	7.63	6.10	0.29	27.2	.20	.20	.20	.20	(5)
F7L	23.7	7.58	6.20	0.35	28.5	.20	.19	.19	.19	(4)
F8U	24.9	7.83	6.78	0.26	25.2	.32	.27	.27	.27	(5)
F8L	24.4	7.82	6.80	0.24	25.0	.26	.24	.23	.23	(6)

Table 3 (continued). ACUTE TOXICITY AND MEAN TEST CONDITIONS IN BIOASSAYS TO DETERMINE VARIABILITY IN RESPONSE OF FATHEAD MINNOWS AND GOLDFISH TO PENTACHLOROPHENOL

Test	Temperature, C	pH meter reading	O_2, mg/l	Weight, g	Length,[a] mm	LC50, mg/l				
						24 hr	48 hr	96 hr	Threshold	(days)
					Goldfish					
G1U	24.2	7.81	5.51	2.36	35.0	.21	.21	.21	.21	(4)
G1L	23.0	7.78	5.66	2.57	36.4	.24	.22	.22	.22	(4)
G2U	25.1	7.77	6.17	1.46	30.2	.23	.23	.23	.23	(4)
G2L	24.3	7.75	6.17	1.50	29.5	.26	.21	.21	.21	(4)
G3U	25.2	7.62	5.86	1.55	29.4	.21	.19	.17	.17	(5)
G3L	24.4	7.68	6.31	1.40	28.7	.18	.17	.17	.17	(4)
G4U	26.2	7.54	5.33	2.46	39.5	.32	.29	.22	.18	(10)
G4L	25.1	7.59	5.71	2.70	39.2	.30	.23	.23	.23	(4)
G5U	25.8	7.58	5.64	1.66	33.5	.32	.30	.24	.21	(10)
G5L	24.6	7.59	5.84	1.74	33.8	.31	.24	.24	.24	(4)
G6U	25.1	7.58	5.39	1.69	33.8	.23	.22	.20	.20	(5)
G6L	24.2	7.60	5.72	1.65	33.7	.22	.19	.19	.19	(4)
G7U	25.0	7.83	6.02	2.31	37.0	.37	.35	.29	.25	(8)
G7L	24.5	7.84	5.72	2.31	37.8	.36	.34	.30	.28	(8)
G8U	24.5	7.73	5.84	1.76	34.8	.24	.21	.20	.15	(11)
G8L	23.8	7.76	6.32	1.54	33.8	.36	.30	.25	.17	(11)

[a] Fatheads - total length; goldfish - standard length.

Table 4. ACUTE TOXICITY AND MEAN TEST CONDITIONS IN BIOASSAYS TO DETERMINE VARIABILITY IN RESPONSE OF FATHEAD MINNOWS AND GOLDFISH TO HEXAVALENT CHROMIUM

Test	Temperature, C	pH meter reading	O_2, mg/l	Weight, g	Length,[a/] mm	LC50, mg/l			
						24 hr	48 hr	96 hr	11 days
				Fathead Minnows					
F1U	24.5	7.65	6.26	0.24	26.9	112	83	56	24
F1L	23.6	7.66	6.73	0.24	27.4	134	95	51	18
F2U	24.7	7.65	6.62	0.20	24.4	160	82	53	17
F2L	23.9	7.67	6.82	0.20	23.9	130	70	49	13
F3U	26.0	7.66	6.05	0.16	22.8	160	92	48	14
F3L	24.9	7.65	6.51	0.16	22.7	180	89	60	12
F4U	24.8	7.58	6.48	0.30	26.9	163	84	50	24
F4L	24.1	7.57	6.99	0.31	27.9	111	79	53	18
F5U	24.8	7.71	6.31	0.29	27.6	108	63	49	21
F5L	24.6	7.70	6.24	0.29	27.3	113	83	37	16
F6U	25.8	7.50	6.27	0.18	24.0	164	144	66	20
F6L	25.1	7.52	6.49	0.18	24.4	182	114	55	22
F7U	25.1	7.62	6.68	0.21	23.1	98	52	38	15
F7L	24.8	7.63	6.79	0.21	23.9	162	61	34	16
F8U	24.7	7.79	6.63	0.37	29.6	110	57	29	19
F8L	23.9	7.78	6.56	0.37	29.6	154	56	34	14

Table 4 (continued). ACUTE TOXICITY AND MEAN TEST CONDITIONS IN BIOASSAYS TO DETERMINE VARIABILITY IN RESPONSE OF FATHEAD MINNOWS AND GOLDFISH TO HEXAVALENT CHROMIUM

Test	Temperature, C	pH meter reading	O_2, mg/l	Weight, g	Length,[a] mm	LC50, mg/l 24 hr	48 hr	96 hr	11 days
					Goldfish				
G1U	24.7	7.64	5.75	2.36	37.8	-	238	123	29
G1L	24.0	7.65	6.11	2.17	36.3	-	202	123	24
G2U	25.8	7.67	6.63	1.64	31.3	259	158	90	24
G2L	24.2	7.66	6.46	1.67	30.4	256	231	125	28
G3U	25.5	7.65	5.96	1.61	30.3	280	236	109	36
G3L	24.6	7.65	6.11	1.49	29.2	-	224	135	59
G4U	25.9	7.67	5.93	1.67	29.7	-	204	110	22
G4L	25.1	7.66	6.06	1.66	29.7	-	-	129	19
G5U	26.1	7.49	5.82	1.85	33.7	256	206	98	45
G5L	25.2	7.47	5.96	1.87	34.9	-	243	133	43
G6U	25.1	7.60	6.19	1.79	34.4	250	208	102	15
G6L	24.6	7.62	6.40	1.61	33.5	-	202	133	18
G7U	24.7	7.76	6.24	2.13	36.3	244	182	126	38
G7L	23.9	7.77	6.21	2.23	37.5	296	205	126	47
G8U	24.6	7.67	5.86	2.30	37.1	245	230	133	43
G8L	24.1	7.68	6.55	2.20	36.8	266	212	126	32

[a] Fatheads - total length; goldfish - standard length.

Table 5. ACUTE TOXICITY AND MEAN TEST CONDITIONS IN BIOASSAYS TO DETERMINE VARIABILITY IN RESPONSE OF FATHEAD MINNOWS AND GOLDFISH TO GUTHION®

Test	Temperature, C	pH meter reading	O_2, mg/l	Weight, g	Length,[a/] mm	LC50, mg/l 24 hr	48 hr	96 hr	11 days
				Fathead Minnows					
F1U	24.9	7.32	6.52	0.27	26.0	5.75	4.33	3.26	0.81
F1L	24.8	7.32	7.58	0.28	26.7	3.75	2.85	2.17	0.83
F2U	24.8	7.25	5.90	0.20	25.2	2.10	1.28	1.06	0.84
F2L	24.7	7.32	5.10	0.19	25.6	2.06	1.87	0.91	0.59
F3U	24.9	7.36	5.95	0.29	27.7	10.02	4.88	1.95	0.69
F3L	24.6	7.37	5.90	0.29	27.7	8.65	6.79	2.17	0.61
F4U	24.6	7.38	7.10	0.38	29.7	6.00	2.45	2.08	0.48
F4L	24.7	7.40	6.80	0.37	29.7	3.53	3.42	0.54	0.18
F5U	24.8	7.23	6.24	0.21	24.6	7.38	3.72	2.53	1.55
F5L	24.4	7.24	6.43	0.22	24.9	4.34	3.80	1.46	0.54
F6U	24.9	7.24	6.96	0.28	27.4	5.70	4.46	2.32	0.72
F6L	24.8	7.21	7.16	0.26	26.1	4.61	3.43	2.47	0.78
F7U	25.0	7.24	6.62	0.20	20.0	11.50	7.01	2.91	0.87
F7L	25.0	7.23	6.85	0.17	19.6	13.70	4.15	1.98	1.76
F8U	24.8	7.39	6.90	0.27	26.4	3.50	1.67	1.20	0.36
F8L	24.6	7.35	6.85	0.27	26.7	2.50	2.03	1.46	0.53

Table 5 (continued). ACUTE TOXICITY AND MEAN TEST CONDITIONS IN BIOASSAYS TO DETERMINE VARIABILITY IN RESPONSE OF FATHEAD MINNOWS AND GOLDFISH TO GUTHION®

Test	Temperature, C	pH meter reading	O_2, mg/l	Weight, g	Length,[a] mm	LC50, mg/l 24 hr	48 hr	96 hr	11 days
					Goldfish				
G1U	24.8	7.20	6.42	2.37	33.9	6.87	5.18	2.23	1.22
G1L	24.4	7.18	6.11	2.51	34.1	8.50	4.61	2.18	1.06
G2U	25.1	7.31	6.15	1.75	30.3	6.98	4.96	2.68	1.13
G2L	25.0	7.31	6.25	1.69	29.6	8.87	6.17	2.45	1.05
G3U	24.7	7.34	6.60	1.87	32.3	5.34	4.20	2.48	1.05
G3L	24.8	7.39	5.00	1.87	32.3	5.35	2.80	1.71	0.47
G4U	24.8	7.12	5.89	1.78	30.7	7.63	5.01	2.07	0.71
G4L	24.4	7.09	5.12	1.75	30.3	11.20	4.03	2.05	0.94
G5U	25.0	7.12	6.99	1.37	32.6	6.81	6.81	2.08	0.73
G5L	24.7	7.11	7.27	1.64	33.6	6.05	4.26	2.13	0.71
G6U	24.9	7.21	6.14	1.72	35.0	-	6.89	3.86	0.83
G6L	24.7	7.20	5.62	1.66	35.3	-	8.75	1.88	0.75
G7U	24.8	7.07	5.65	1.81	37.9	10.60	5.58	3.02	0.19
G7L	24.6	7.13	6.62	1.80	36.0	7.18	3.69	2.05	0.43
G8U	24.6	7.18	5.66	3.50	42.4	9.70	8.36	1.35	0.90
G8L	24.4	7.16	6.19	3.53	41.5	-	5.37	3.76	0.71

[a] Fatheads - total length; goldfish - standard length.

Table 6. TEST CONDITIONS IN BIOASSAYS WITH LARGE AND SMALL FATHEAD MINNOWS OF CONSTANT AGE

	Fish weight, g		Fish length, mm[a/]		Temperature, C		pH meter reading		Oxygen, mg/l	
Test	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range
F1A	.09	.037	16.6	2.37	24.8	23.5-25.6	7.83	7.67-7.99	6.7	6.5-7.1
F1B	.27	.094	23.8	2.61	24.8	24.0-25.9	7.81	7.64-7.98	6.8	6.6-7.1
F2A	.11	.038	16.5	2.07	25.0	23.9-25.5	7.70	7.65-7.85	7.0	6.8-7.3
F2B	.28	.094	23.5	2.51	24.2	23.6-24.5	7.68	7.64-7.71	7.1	6.8-7.3
F3A	.05	.019	16.8	1.71	24.9	24.0-25.5	7.74	7.64-7.92	6.7	6.4-7.2
F3B	.26	.090	28.8	2.97	24.6	24.0-25.6	7.75	7.69-7.91	6.7	6.5-7.0

[a/] Total length.

Table 7. TEST CONDITIONS IN BIOASSAYS WITH FATHEAD MINNOWS OF VARIOUS AGES

Test	Age, wk	Fish weight, g		Fish length, mm[a/]		Temperature, C		pH meter reading		Oxygen, mg/l	
		Mean	SD	Mean	SD	Mean	Range	Mean	Range	Mean	Range
F4A	4	.02	.007	13.2	1.59	25.3	25.0-25.7	7.72	7.67-7.86	6.6	6.4-7.0
F5A	4	.03	.014	14.3	1.92	24.6	24.3-25.1	7.81	7.71-7.96	6.7	6.5-7.0
F6A	4	.01	.011	12.1	2.55	24.2	23.5-24.6	7.84	7.84-8.03	6.7	6.6-6.9
F7A	4	.02	.008	12.8	1.94	25.2	24.5-26.0	7.72	7.68-7.74	6.7	6.6-7.0
F4B	7	.04	.025	17.6	1.99	23.7	23.3-24.3	7.71	7.65-7.87	6.7	6.5-7.1
F5B	7	.10	.036	20.8	2.55	24.7	24.5-25.0	7.86	7.80-7.98	6.9	6.8-7.1
F6B	7	.05	.034	17.1	2.88	25.2	24.5-25.7	7.88	7.82-8.02	6.7	6.1-7.0
F7B	7	.03	.011	15.0	1.48	24.6	23.8-25.3	7.77	7.69-7.97	6.8	6.6-7.1
F4C	11	.15	.065	24.7	3.38	24.9	24.6-25.2	7.80	7.74-7.88	6.8	6.6-7.0
F5C	11	.24	.099	27.4	3.49	23.7	23.1-24.3	7.83	7.72-8.03	6.8	6.7-7.0
F6C	11	.16	.072	25.1	4.01	25.5	25.0-25.7	7.84	7.67-8.02	6.5	6.3-6.7
F7C	11	.21	.090	25.8	3.63	25.2	24.3-25.5	7.76	7.70-7.93	6.6	6.4-6.9
F4D	14	.21	.047	27.8	2.16	25.0	24.3-25.4	7.70	7.63-7.83	6.5	6.3-7.0
F5D	14	.29	.123	29.6	4.13	24.5	23.9-25.1	7.82	7.72-8.01	6.7	6.4-7.1
F6D	14	.20	.093	26.6	4.15	25.3	24.5-25.6	7.82	7.65-8.07	6.5	6.0-7.0
F7D	14	.32	.117	30.9	3.61	25.2	24.5-26.0	7.72	7.68-7.74	6.7	6.6-7.0

[a/] Total length.

Table 8. TEST CONDITIONS IN BIOASSAYS WITH LARGE AND SMALL GOLDFISH FROM A CONSTANT STOCK

	Fish weight, g		Fish length, mm[a/]		Temperature, C		pH meter reading		Oxygen, mg/l	
Test	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range
G1A	1.41	.336	34.8	3.23	25.2	24.5-26.1	7.74	7.60-7.85	5.1	4.6-5.6
G1B	2.68	.652	42.8	3.04	25.2	24.2-25.8	7.80	7.63-8.00	6.0	5.6-6.3
G2A	1.62	.455	35.8	3.04	25.4	24.7-25.7	7.70	7.63-7.80	5.7	5.4-6.1
G2B	3.15	.751	44.2	3.43	25.4	24.6-25.6	7.71	7.60-7.85	6.2	6.0-6.4
G3A	1.70	.426	34.4	2.50	25.4	24.8-26.2	7.70	7.62-7.83	4.8	4.0-6.0
G3B	3.59	.838	43.3	3.10	25.5	24.9-26.2	7.75	7.69-7.91	6.0	5.6-6.6

[a/] Standard length.

the desired concentration was immediately attained and results compared to the previous series. Goldfish were acclimated for at least 10 days at 21 C and for 3 days at the 25 C test temperature. Fathead minnows were reared at a constant 25 C. Photoperiod during the test consised of 12 hr of light and 12 hr of dark. Fish in all tests were fed a small amount of Glencoe pelleted trout food each day, and once per day debris was siphoned from the aquaria.

Deaths were recorded as each fish died during the first 8 to 12 hours and at 24-hour intervals thereafter. Tests were conducted for 11 days or until no additional mortality occurred during a 48-hour period, whichever came first. Lethal concentration to 50% (LC50) was determined graphically on semi-logarithmic paper (APHA, 1971), and the LC50 computed at the start of a 2-day period without mortality was used as the criterion for achieving a threshold LC50. With hexavalent chromium and Guthion® a threshold LC50 was not usually attained by 11 days, but technical limitations made it necessary to terminate tests at that time.

Effect of Size and Age--Three series of experiments were conducted: 1) effect of size with a constant stock of goldfish (Table 8); 2) effect of size with constant aged fathead minnows (Table 6); and 3) effect of different aged fathead minnows (Table 7). A single stock of fish was used for all goldfish experiments. For each individual test 160 fish were randomly selected from the stock, and these were divided visually into groups of 80 large and 80 small fish. The two groups were then assigned to bioassay aquaria in a stratified random manner with 10 fish per aquarium. In the series of experiments for determination of size effect with fathead minnows, constant aged (11-week) fish were selected from the culture unit on three occasions. Each group was separated into large and small fish and then tested in the same manner as the goldfish. In the four experiments for the determination of the effect of age, fathead minnows of 4, 7, 11, and 14 weeks were removed from the culture unit; very small, large or malformed individuals were discarded, and each group was sorted into the four sets of test chambers in a stratified

random manner with 10 fish per chamber. The bioassay procedures and computations of LC50's were the same as described above except that logarithmic-probability graph paper was used for graphical determination of LC50's.

Chemical Analysis

In the series of tests for determination of bioassay variability, concentrations of toxicant in each test chamber were analyzed at 2- or 3-day intervals. In addition, composite samples of pentachlorophenol were accumulated twice a day for the last five series of tests and analyzed at the end. A comparative analysis of fresh and stored samples indicated no deterioration of toxicant. In the series of tests for determination of effect of size or age, only composited samples of pentachlorophenol were anlayzed at the end of each test.

Haxavalent chromium was analyzed colorimetrically [method 211(II)D] without filtration since a filtered and unfiltered analysis gave the same result. Chloride was analyzed by mercuric nitrate titration (method 112B) and results were reported as sodium chloride (APHA, 1971).

In the first three series of tests for determination of bioassay variability, pentachlorophenol was analyzed colorimetrically after addition of Safranin-O and extraction into chloroform (Haskins, 1951). A standard curve was prepared by analysis of known quantities of reagent grade pentachlorophenol dissolved in water and extracted and analyzed in the same manner as the unknown samples. In the last five series of bioassays for determination of variability and in all tests for determination of effect of size and age, pentachlorophenol was extracted into benzene, acetylated and analyzed on a Beckman GC 72-5 gas chromatograph with a 122 cm glass column packed with 5% DC 200 on 80/100 mesh Gas Chrom-Q and a nonradioactive electron capture detector (Rudling, 1970). Comparison of the colorimetric and gas chromatographic methods indicated no difference. Standard curves were prepared by solution of reagent grade pentachlorophenol into benzene, acetylation and measurement of

peak height on the gas chromatograph. Due to non-linearity of the standard curve, unknowns were diluted so that all samples fell in the narrow range of 0.05 to 0.15 mg/liter pentachlorophenol. A control water sample spiked with a known quantity of reagent grade pentachlorophenol was analyzed with each group of unknowns. The mean recovery efficiency of the spike for the series of tests determining variability was 93.7% (standard deviation 4.73) and for the tests of size or age effect, mean recovery efficiency was 86.9% (standard deviation 7.13). Unknowns were corrected by the mean recovery efficiency for each bioassay.

After extraction into benzene, Guthion® was analyzed with the same gas chromatograph parameters as pentachlorophenol. Again, due to non-linearity of the standard curve, samples from bioassay chambers were diluted so that the final concentration in benzene was 1 to 3 mg/liter. Standards were prepared by solution of the 93% technical grade Guthion® into benzene in that range of concentrations. Since control water was contaminated, a deionized water sample was spiked with a known concentration of Guthion® in acetone and was analyzed along with each group of unknowns. Mean recovery efficiency was 95.6% (standard deviation 6.10), and unknowns were corrected for the 93% technical grade standards and the recovery efficiency for each bioassay.

Dissolved oxygen concentrations, pH, temperature, and total alkalinity were monitored routinely during each test by the following methods: oxygen, polarographic meter; pH, meter; temperature, thermister; total alkalinity, titration with brom-cresol green indicator.

Experimental Design and Statistical Analysis

Determination of Variability--Eight tests were conducted in duplicate with each species and each toxicant over a 2-year period to determine the variability of bioassay results. Each of eight different shipments of goldfish was tested with the four toxicants and different groups of 11-week-old fathead minnows were tested with all toxicants. This

experimental design permitted a determination of the coefficient of variation (CV) for the 24-hour, 96-hour, and threshold or 11-day LC50's for each toxicant with each species. Since each individual bioassay was conducted in duplicate, multiway analyses of variance of the LC50's were also performed to determine significant differences between different stocks of fish and between the two sets of bioassay chambers in which the duplicates were conducted. To estimate the probability of an uncontrolled variable affecting the LC50's with different fish stocks, a modification of the standard multiway analysis of variance was used where error due to the slope of individual treatments against their deviation from the mean response was partitioned (Mandel, 1961).

Since eight analyses were performed at each time interval, a rigid per comparison error rate of 0.0064 was computed by the formula:

$$\alpha = 1 - (1-P)^{1/n}$$

where α = the probability of making a Type I error
P = probability of occurrence of an error
n = number of analyses

This reduced the possibility of making at least one Type I error by chance in rejection of the null hypothesis (no significant differences between stocks, duplicate tests, or slopes). The per comparison error rate of 0.0064 was equivalent to a per experiment error rate of 0.05 with the experiment consisting of eight analyses of variance at each time interval (two species, four toxicants).

Determination of Effect of Size and Age--Toxicity curves (LC50 versus time) were drawn for comparison of the sensitivity of small versus large goldfish and fathead minnows and for various aged fathead minnows. The

curves were drawn from the combined data of all bioassays with each species within a size or age group. These hyperbolic curves were transformed to straight lines by plotting the reciprocal of the LC50 versus the reciprocal of time to permit statistical comparison. Analysis of covariance or t-tests were then calculated to examine differences in slope and elevation of the straight lines.

GOLDFISH CULTURE STUDY

Apparatus

During most of the experimental period the 16 test chambers used were constructed of 19 mm thick exterior plywood coated on the inside with Sears polyester finishing resin. Eight of the tanks measured 61 x 46 x 46 cm and eight others measured 122 x 46 x 46 cm. A polyvinyl chloride standpipe at one end of each tank maintained the water depth at 34 cm so that the small tanks contained 95 and the large tanks 190 liters. Each tank contained an air diffusion stone, use of which was initiated 2 months after the start of the experiment. The tanks were spacially arranged in a stratified random manner to accomodate the 2^3 factorial design of the experiment (Figure 1).

In the initial stages of the experiment eggs were hatched in a two-chambered glass container. The incoming water flowed into a 20 x 20 x 10 cm deep chamber and then through a perforated glass tube into a 10 x 10 x 5 cm deep compartment. The eggs were not moved by the flow of water and movement of dye indicated that water flowed evenly over the eggs. Hatched fry were caught in a 7.5 x 7.5 x 10 cm deep basket as they came through the overflow tube. This basket had three glass sides and bottom and one Nitex (nylon) screened side and was immersed in a 22-liter glass aquarium measuring 50 x 25 x 26 cm deep. The same well water as used in flow-through acute bioassays (Table 1) was supplied to the to the test chambers via two modified proportional diluters constructed of glass, G.E. Silicone Seal, and Koroseal tubing (Mount and Brungs, 1967). Each diluter consisted of only the upper distribution system for delivery of fresh water. The eight chambers in this section

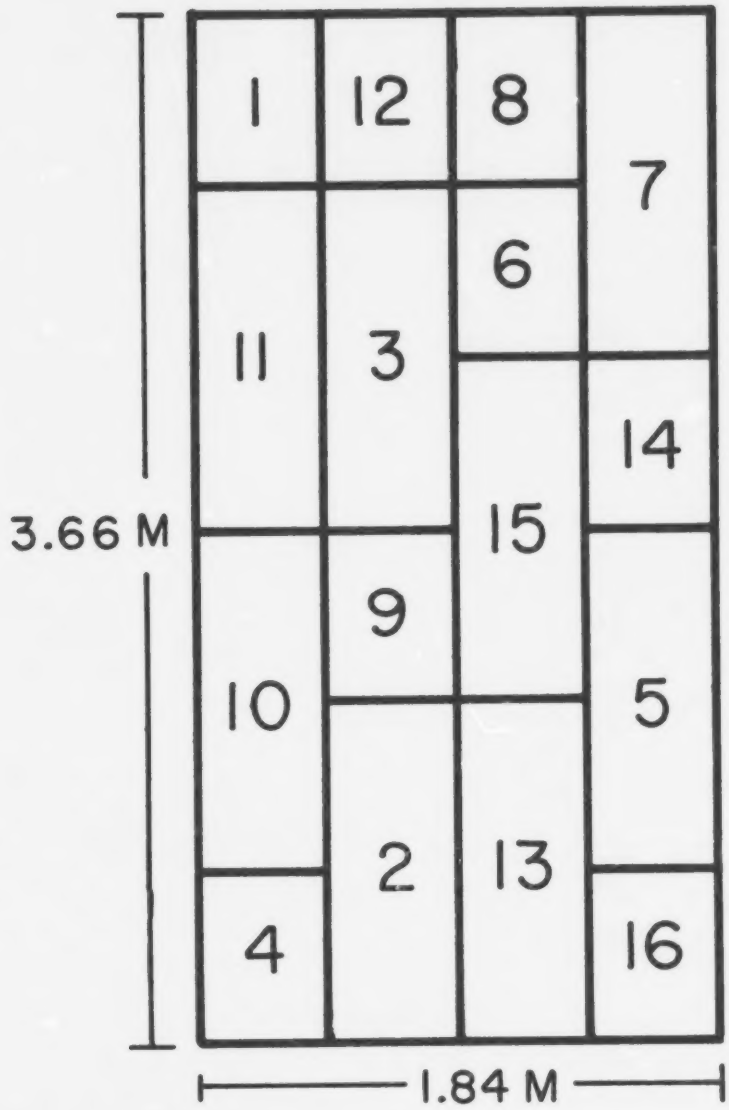

Figure 1. Spatial arrangement of test chambers drawn to scale in goldfish culture study. Conditions in each chamber are detailed in Table 19.

alternated in size with four dispensing 500 ml to the smaller tanks and four delivering 1000 ml to the large tanks. Water from a ninth chamber, normally used for delivery of control water in toxicant dispensing systems, ran to the drain since maintenance of a precise volume was difficult. With a cycle time of 2 minutes 10 seconds, 90% of the water in both small and large tanks was replaced in approximately 15 hours. Water was supplied to each diluter from a separate head tank containing a stainless steel hot water heat exchange system controlled by a thermostat. The pH was effectively controlled by a chemical metering device (Mount and Brungs, 1967) dispensing sulfuric acid into the head tank along with the incoming water.

Six fluorescent light fixtures, each holding two 40-watt cool-white fluorescent tubes, were suspended 55 cm above the water surface and were arranged so that all tanks received approximately equal illumination. Photoperiod throughout the experiment was 16 hours light and 8 hours dark.

Experimental Design

The experiment was arranged in a 2^3 factorial design with two replications (Figure 1). The three treatments were temperature, nominally 20 and 25 C; fish density, same number in large and small tanks; and food, Oregon moist pelleted trout food and Oregon moist supplemented with a variety of other foods. One-way analysis of variance was used for determining significant differences due to each factor as well as the two and three factor interactions. Since one tank of fish was lost due to an accident, the average for the replicate tank was used to supply the missing data, and one degree of freedom was subtracted from the error term.

The effects of the three factors on survival, growth, and maturation were measured. If maturation occurred, then observations would have been made on the presence or absence of spawning, fecundity, and time till initiation of spawning.

Procedure

A group of 4-year-old goldfish from Ozark Fisheries, Inc. that had been held in our laboratory at approximately 11 C for about 18 months was used as brood stock for obtaining eggs. These fish had been induced to spawn many times by raising the water temperature to 20 C over a period of 2 days. On the third day a portion of the stock would spawn. With this procedure eggs from three ripe females were hand stripped into three Petri dishes; milt from excised testes of three ripe males was squeezed through cheesecloth onto the eggs; and water at 22 C was added and the mixture swirled for 2 minutes to complete fertilization. From each spawning pair eggs were randomly sorted into two groups of 200 eggs. One group from a spawning pair was placed in an egg hatching chamber at 20 C and one at 25 C after the Petri dish was floated on the surface of the appropriate water until temperature equilibrated.

Six days after the start of the experiment all healthy fry from each temperature were consolidated into two 22-liter aquaria at the respective temperature. Thirteen days later fish within each temperature group were assigned in a stratified random manner to each of the wooden tanks, 18 per tank, after removal of the largest, smallest, and obviously deformed fish. After 42 days of the experiment fish were thinned to 12 per tank and after 122 days to 10 per tank with prior removal of largest, smallest, and unhealthy or deformed fish.

After 2-1/2 months and at monthly intervals thereafter fish were not fed for 24 hours, and on the next day fish from each tank were weighed as a group in water on a triple beam balance. At termination after 393 days weights and standard lengths of individual fish were taken, fish were sexed, and a judgment was made on the state of maturation of the gonads using a Roman numeral classification system.

Food

The quantity and quality of the food was changed at various times depending on the size of the fish and the availability of certain foods.

At all times fish were fed in excess with equal amounts given to each tank. For the first 43 days all fish received the same diet, and for the remainder of the experiment one group received only Oregon moist pelleted trout food and the other group received proportionally less Oregon moist supplemented by other foods indicated in Table 9. Other foods used were Glencoe dry trout pellets, ground for consumption by fry; pulverized hard-boiled chicken egg yolk; live brine shrimp nauplii and adults; a mixture of fresh lettuce and fathead minnows ground in a blender; chopped frozen spinach; live earthworms; and fresh duckweed (Lemna minor) and various incidental organisms associated with it.

Monitoring Test Conditions

Temperature, pH, and dissolved oxygen concentrations were recoreded once per week for each tank, and total alkalinity once per week for each temperature group. Cycle time for each diluter was measured twice each week with adjustments made as necessary to attempt to maintain a 2-minute interval.

Temperature in each tank was measured with a YSI Telethermometer and each diluter was also monitored by a continuously recording Honeywell 24-channel recorder. Total alkalinity, pH, and dissolved oxygen were analyzed as described in the acute bioassay section.

Table 9. DIET OF TWO GROUPS OF GOLDFISH DURING DIFFERENT PERIODS OF THE EXPERIMENT[a/]

Duration, days	Daily schedule	Straight diet	Mixed diet
1-19	AM-1	Egg yolk	Egg yolk
	AM-2	Glencoe	Glencoe
	PM-1	Glencoe	Glencoe
	PM-2	Brine shrimp	Brine shrimp
20-43	AM-1	Glencoe	Glencoe
	AM-2	Fish - lettuce	Fish - lettuce
	AM-3	Egg yolk	Egg yolk
	PM-1	Fish - lettuce	Fish - lettuce
	PM-2	Brine shrimp	Brine shrimp
44-161	AM-1	Oregon moist	Fish - lettuce
	PM-1	Oregon moist	Oregon moist
	PM-2	Oregon moist	Oregon moist
162-205	AM-1	Oregon moist	Spinach
	AM-2	--	Oregon moist
	PM-1	Oregon moist	Oregon moist
	PM-2	Oregon moist	Earthworms
206-327	AM-1	Oregon moist	Duck weed
	AM-2	--	Oregon moist
	PM-1	Oregon moist	Oregon moist
	PM-2	Oregon moist	Duck weed
328-391	AM-1	Oregon moist	Spinach
	AM-2	--	Oregon moist
	PM-1	Oregon moist	Oregon moist
	PM-2	Oregon moist	Spinach

[a/] Consult text for description of the food indicated.

SECTION V

RESULTS

ACUTE TESTS

Acute Toxicity

Sodium chloride—Mean dissolved oxygen concentrations for all tests were 7.05 and 6.87 mg/liter, mean pH meter readings 7.23 and 7.11, mean temperatures 25.0 and 24.9 C (Table 2), and mean total alkalinities 87 and 83 mg/liter $CaCO_3$ (ranges 80-100 and 73-91) for fathead minnows and goldfish, respectively.

Most mortality from sodium chloride occurred within the first 48 hr after which the toxicity curves paralleled the abscissa (Figure 2). Goldfish were initially more resistant than fathead minnows but after 48 hr became significantly less resistant with a threshold LC50 of 7322 mg/liter for goldfish and 7650 mg/liter for fathead minnows ($t = 2.159$, $p = .02$-$.05$) (Table 10).

Immediately upon introduction of sodium chloride both species were affected even at concentrations that caused no mortality during the tests. For about the first 5 min of exposure there was increased swimming activity particularly toward the water surface. After this the fish settled into a pattern of normal activity and food searching near the aquarium bottom. Within 1 to 2 hr prior to death fish began increased respiratory movements at the surface accompanied by occasional bursts of frenzied swimming. Just prior to death swimming ceased and opercular movements slowed considerably. Death was presumed to be due to massive

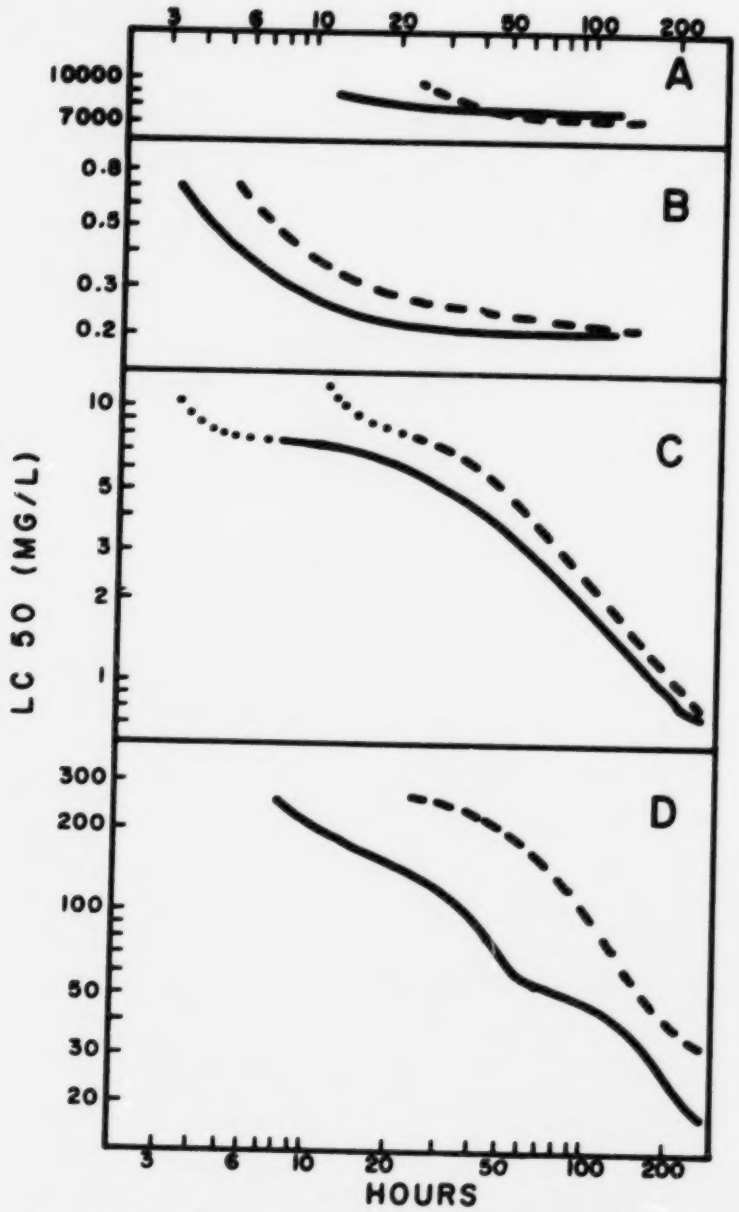

Figure 2. Toxicity curves of (A) sodium chloride, (B) pentachlorophenol, (C) Guthion, and (D) hexavalent chromium for goldfish (dashed lines) and fathead minnows (solid lines). At times of 24 hr or longer curves are visually fitted through mean LC50's from at least 14 tests and at earlier times through mean LC50's from at least 8 tests. Hypothesized extensions of the Guthion curves based on limited data are represented by dots.

osmoregulatory failure.

Pentachlorophenol—Mean dissolved oxygens were 6.54 and 5.82 mg/liter, mean pH meter readings 7.72 and 7.69, mean temperatures 24.8 and 24.7 C (Table 3), and mean total alkalinities 211 and 211 mg/liter $CaCO_3$ (ranges 204-216 and 206-217) for fathead minnows and goldfish, respectively.

Toxicity curves for pentachlorophenol were similar to those for sodium chloride in that initial mortality was rapid and the curves became parallel to the abscissa by 48 hr for fathead minnows and by about 96 hr for goldfish (Figure 2). Goldfish were initially more resistant but there was no significant difference in the threshold LC50 of 0.21 mg/liter for both species ($t = .117$, $p > .5$) (Table 10).

Fish exposed to pentachlorophenol seemed unaffected until a few hours before death. They then exhibited increased swimming activity near the surface, followed by quiescence, then death at the bottom of the test chamber. Webb and Brett (1973) list toxic effects of pentachlorophenol and classify it as a general metabolic stressor, uncoupling oxidation phosphorylation.

Guthion®—Mean dissolved oxygen concentrations were 6.55 and 6.09 mg/liter, mean pH meter readings 7.30 and 7.20, mean temperatures 24.8 and 24.7 C (Table 5), and mean total alkalinities 89 and 87 mg/liter as $CaCO_3$ (ranges 82-93 and 81-92) for fathead minnows and goldfish, respectively.

The toxicity curves (Figure 2) for Guthion® are similar in shape for both species but differ from the curves for sodium chloride and pentachlorophenol as well as typical curves for many other toxicants as reported by Sprague (1969). The curves are nearly parallel to the abscissa at 6 to 12 hr for fathead minnows and at 18 to 26 hr for goldfish. This result is presumed to be an artifact resulting from concentrations too low to cause measurable mortality in less than 6 hr for fathead minnows and 18 hr for goldfish. Individual points used to construct these curves

Table 10. MEANS AND 95% CONFIDENCE INTERVALS OF LC50'S (MG/LITER) OF FOUR TOXICANTS AND TWO FISH SPECIES AT VARIOUS TIMES

	NaCl		PCP		Guthion®		Cr^{+6}	
Hours	FH[a/]	GF[b/]	FH	GF	FH	GF	FH	GF
3	--	--	.70 ±.07	--	--	--	--	--
4	--	--	.55 ±.04	--	--	--	--	--
5	--	--	--	.69 ±.08	--	--	--	--
8	--	--	.38 ±.03	.46 ±.05	7.43 ±2.94	--	257 ±40	--
12	8530 ±663	--	.25 ±.03	.33 ±.03	6.82 ±1.96	--	195 ±14	--
24	7910 ±330	9952 ±365	.22 ±.02	.27 ±.03	5.94 ±1.87	7.78 ±1.14	140 ±15	261 ±13
48	7691 ±257	7706 ±308	.21 ±.02	.24 ±.03	3.63 ±.90	5.42 ±.87	82 ±13	212 ±13
72	7650 ±234	7388 ±236	.21 ±.02	.24 ±.03	2.31 ±.50	3.65 ±.42	56 ±8	167 ±15
96	7650 ±234	7341 ±234	.21 ±.01	.22 ±.02	1.90 ±.40	2.37 ±.36	48 ±6	120 ±8
120	7650 ±234	7322 ±224	.21 ±.01	.21 ±.02	1.51 ±.33	1.99 ±.32	43 ±5	83 ±10
144	--	7322 ±224	--	.21 ±.02	1.25 ±.30	1.44 ±.23	38 ±5	65 ±12
168	--	--	--	--	1.06 ±.25	1.25 ±.23	32 ±3	53 ±9

Table 10 (continued). MEANS AND 95% CONFIDENCE INTERVALS OF LC50'S (MG/LITER) OF FOUR TOXICANTS AND TWO FISH SPECIES AT VARIOUS TIMES

	NaCl		PCP		Guthion®		Cr^{+6}	
Hours	FH	GF	FH	GF	FH	GF	FH	GF
192	--	--	--	--	.95	1.15	27	45
					±.23	±.24	±2	±9
216	--	--	--	--	.81	1.02	23	39
					±.21	±.18	±2	±7
240	--	--	--	--	.78	.92	20	34
					±.22	±.17	±2	±7
264	--	--	--	--	.76	.80	18	33
					±.22	±.15	±2	±7

a/ Fathead minnow.
b/ Goldfish.

were the means of at least 14 LC50 values at times of 24 hr or longer and means of at least 8 LC50 values at times earlier than 24 hr. In two tests with goldfish where test concentrations were set somewhat higher and early mortality occurred, the mean 8-hr LC50 was 17 mg/liter and the mean 4-hr LC50 from two fathead minnow tests under similar conditions was 8.5 mg/liter. As indicated by the dotted line (Figure 2), it is hypothesized that these curves bend steeply upward at shorter time intervals. The resultant change in slope throughout the curves may indicate different mechanisms of toxic action or at least a period of physiological adjustment before further mortality occurs. The toxic effect of Guthion® and its oxidized metabolite is inhibition of acetylcholinesterase (Chemagro Division Research Staff, 1974).

As with sodium chloride and pentachlorophenol, goldfish were initially more resistant, but at 11 days the LC50's were not significantly dif-

ferent with 0.76 mg/liter for fathead minnows and 0.80 mg/liter for goldfish ($t = .381$, $p>.5$) (Table 10). At 11 days threshold LC50 values had not been attained for goldfish, but the toxicity curve for fathead minnows was just starting to parallel the abscissa (Figure 2).

Early mortality of fathead minnows was unusual in that deaths were poorly related to toxicant concentration. In typical tests the following percentage mortalities had occurred after 1 hr:

Concentration (mg/l):	0	0.5	0.9	1.6	2.5	4.8	6.8	10.0
Percentage mortality:	0	20	0	0	20	40	90	40

Upon introduction of the Guthion®, fish in all concentrations would appear stressed within 5 min, many lost equilibrium, and some died as indicated by the results above. After the initial mortality the death rate decreased for up to 24 hr and then again increased (Figure 2). Fish that lost equilibrium would either die almost immedaitely or would lie on the bottom of the aquarium in this condition for a few days before death occurred. This loss of equilibrium in the environment would be essentially equivalent to death since the individual is immediately vulnerable to predators and is at the mercy of water currents. Therefore, LC50 is not a good predictor of environmental damage. EC50's based on loss of equilibrium would be lower than LC50's at the same time intervals.

Hexavalent Chromium--Mean dissolved oxygen concentrations from all tests were 6.53 and 6.14 mg/liter, mean pH meter readings 7.65 and 7.64, mean temperature 24.7 and 24.9 C (Table 4), and mean total alkalinities 214 and 214 mg/liter $CaCO_3$ (ranges 200-230 and 200-222) for fathead minnows and goldfish, respectively.

The toxicity curves for hexavalent chromium are somewhat unusual when compared to typical hyperbolic curves of other toxicants (Figure 2). The shape of the curve for goldfish is similar to the Guthion® curves and limited data obtained for earlier times suggest that it bends sharply upward at the shorter time intervals. The curve for fathead

minnows indicates an initial period of rapid mortality, followed by a period of reduced mortality from 72 to 120 hr, and then again increased mortality. This curve suggests the possibility of two modes of toxic action, one at high and one at lower chromium concentrations. Grindley (1946) reported a similar curve showing the effect of potassium dichromate on rainbow trout. However, this test was conducted at considerably higher concentrations of chromium. In a series of studies on the toxic effect of hexavalent chromium it was found that the primary path of entry was by diffusion through the gills of rainbow trout (Knoll and Fromm, 1960), that no gill pathology occurred but the epithelial cell lining of the intestinal tract of largemouth bass was sloughed off (Fromm and Schiffman, 1958), and that glucose uptake by epithelial cells of the intestinal tract was inhibited (Stokes and Fromm, 1965). These authors did not suggest more than one mode of toxic action. Since glucose inhibition was found in live fish exposed to 2.5 mg/liter hexavalent chromium for 7 days, this may be the mode of toxic action at times longer than 72 hr. The cause of death at earlier times is unknown if another mode of toxic action exists.

With the other three toxicants the goldfish were initially more resistant, but by the end of the test, LC50's were the same for both species (pentachlorophenol and Guthion®) or less for goldfish (sodium chloride). With hexavalent chromium the goldfish were more resistant throughout the entire test. The 11-day LC50 of 33 mg/liter for goldfish was significantly greater than that of 18 mg/liter for fathead minnows ($t = 4.601$, $p = .01$-$.02$) (Table 10). Unless these species differ considerably in their resistance to chromium as compared to the other toxicants, it would be expected that after 11 days the chromium toxicity curve for goldfish would bend downward resulting in a curve similar to that for fathead minnows.

Since initial chromium concentrations were gradually increased to the desired concentrations in most tests, three tests on each species were conducted to compare the difference in LC50's when test concentrations were attained immediately. The toxicity curves for both species were

similar in shape to the curves obtained by the first experimental procedure (Figures 2 and 3). The LC50's for fathead minnows were lower at all times, but the LC50's for goldfish were higher after 24 hr (Tables 10 and 11). The difference in procedure affected the overall acute mortality of fathead minnows, and the 11-day LC50 of 18 mg/liter by the first procedure was significantly higher than the 11-day LC50 of 12 mg/liter ($t = 2.482$, $p = .02$-$.05$) by the second procedure. There was no significant difference in overall toxicity to goldfish using the two procedures with 11-day LC50's of 0.33 and 0.38 mg/liter ($t = .722$, $p > .5$).

The onset of detrimental effects was usually observable from 24 to 48 hr before death. Respiratory movements became more rapid and pronounced and fish surfaced frequently. Prior to death respiratory movements slowed, fish lost equilbrium, and they died shortly thereafter. Within 24 hr after the introduction of even the lowest chromium concentrations tested, the bottom of the goldfish chambers became littered with tapeworms.

Variability of Test Species

In the previous section mortality of the fish due to the four toxicants was discussed primarily in terms of toxicity curves depicting death rates during the entire test. Standard bioassays for comparative purposes by industrial or research laboratories will probably continue to report results as LC50's at fixed times, most likely 24 hours as with the ORSANCO (1974) bioassay or 96 hours as with the proposed Canadian procedure (MacLeod, 1972). Reporting of threshold LC50's has become more common where these values are attained in a relatively short time. Therefore, the comparison of variability described here is in terms of 24- and 96-hr LC50's for all toxicants as well as threshold LC50's for sodium chloride and pentachlorophenol and 11-day LC50's for hexavalent chromium and Guthion® with which threshold values were not attained by termination of the bioassay. Table 12 lists the mean LC50 values for

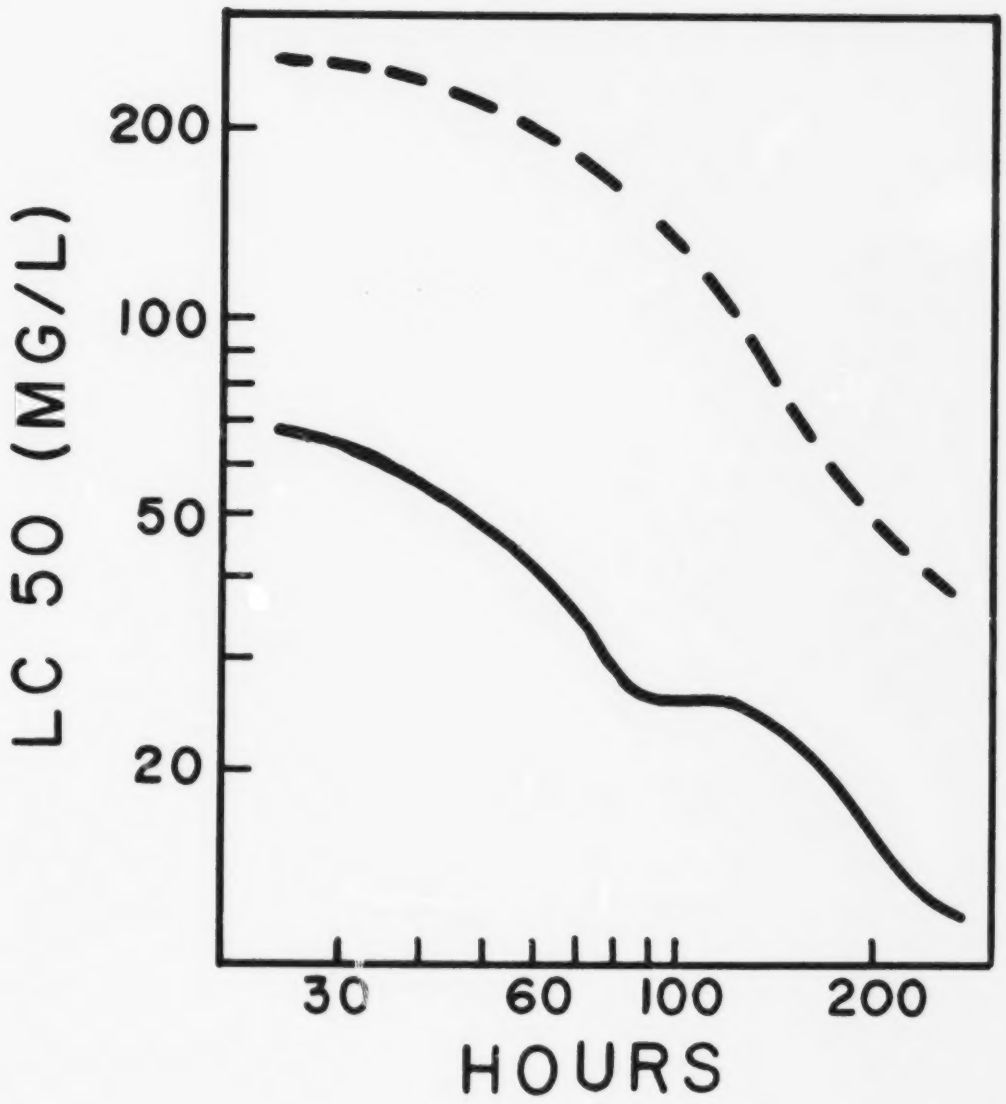

Figure 3. Toxicity curves of hexavalent chromium for goldfish (dashed line) and fathead minnow (solid line). Curves are visually fitted through mean LC50's from three tests using the second experimental procedure.

Table 11. MEANS AND 95% CONFIDENCE INTERVALS OF LC50'S OF HEXAVALENT CHROMIUM FROM RESULTS OF THE SECOND EXPERIMENTAL PROCEDURE

Hours	LC50, mg/1 Cr^{+6}	
	Fathead	Goldfish
24	66 ±18	245 ±62
48	50 ±12	213 ±49
72	33 ±21	188 ±57
96	26 ±11	124 ±33
120	26 ±11	101 ±35
144	24 ±12	76 ±69
168	20 ±12	60 ±18
192	18 ±12	53 ±17
216	14 ±13	45 ± 5
240	13 ±12	40 ±18
264	12 ± 8	36 ±17

Table 12. MEAN LC50'S (MG/LITER) WITH COEFFICIENT OF VARIABILITY (PERCENTAGE) OF FOUR TOXICANTS AT VARIOUS TIMES

	Time					
	24 hr		96 hr		Terminal[a/]	
Toxicant	LC50	CV	LC50	CV	LC50	CV
			Fathead Minnow			
NaCl	7910	8	7650	6	7650	6
PCP	0.22	19	0.22	12	0.21	12
Cr^{+6}	140	20	48	22	18	21
Guthion	5.94	58	1.90	39	0.76	52
			Goldfish			
NaCl	9952	7	7341	6	7322	6
PCP	0.27	23	0.22	17	0.21	16
Cr^{+6}	261+	--[b/]	120	12	33	38
Guthion	7.78	24	2.37	29	0.80	35

[a/] Threshold LC50 for NaCl and PCP, 11-day LC50 for Cr^{+6} and Guthion.
[b/] 24-hr LC50 not obtained in all tests due to slow mortality.

the four tested materials at these times along with the coefficient of variation. Since the LC50's of the toxicants differ by up to four orders of magnitude, easy comparison of variability by standard deviation is impossible without normalization to the coefficient of variation.

The LC50 values of sodium chloride were the least variable of the four toxicants at all times, with essentially no difference between fathead minnows and goldfish. Pentachlorophenol ranked second although with this toxicant goldfish were slightly more variable. Hexavalent chromium and Guthion® ranked third and fourth, respectively, although missing data and some variation at different times make this ranking less definitive. With sodium chloride and pentachlorophenol there was little difference in variability between 96-hr and threshold LC50's, but variability at 24 hours was greater than at the other times. With hexavalent chromium there was essentially no difference in variability between 24-, 96-hr, and 11-day LC50's with fathead minnows but with goldfish the variability of the 96-hr LC50 was considerably less than at 11 days, and because initial mortality was slow too few 24-hr LC50's were determined for computation of a reliable coefficient. The fathead minnows had minimum variability to Guthion® at 96 hours and similar variability at 1 and 11 days, whereas the goldfish showed increasing variability at 1, 4, and 11 days. In general, the 96-hr measurement was the least variable, but where threshold values were attained, they showed similar variability.

Neither fish species showed consistent superiority in minimal variability. Both were the same for sodium chloride. The fathead minnows were slightly less variable with pentachlorophenol and considerably more variable with Guthion®. With chromium the goldfish were less variable at 96 hours, more variable at 11 days, and no comparison was available at 24 hours.

The analyses of variance indicated only one instance of a significant difference between the replicate bioassay apparatus used for testing each stock. That difference occurred with goldfish at 96 hours testing hexavalent chromium (Table 1). Since there was also a significant

difference in slope, it appeared that some independent variable was responsible for the difference between results from the two types of apparatus. Correlation coefficients were computed for the LC50's and all quantifiable independent variables, which included weight, length, condition factor, age of fish, time of pre-bioassay holding, dissolved oxygen concentration, pH, and temperature. Only temperature correlated ($r = -.69$). An anlysis of covariance with temperature as the covariate was then computed and the results from the two bioassay apparatus were still significantly different, indicating that temperature was not the cause of the difference. Since slope was not significantly different in any other comparison, there was no inidication of an independent variable affecting the LC50's.

Reference Toxicant

The same data used to evaluate the variability of the two fish species also indicate the variability of the toxicants. Sodium chloride bioassays were considerably less variable at all times (Table 12). Therefore this compound best meets the criterion of minimum variability in response of the fish to a reference toxicant. Pentachlorophenol ranked second in variability, and although the coefficient of variation was about two or three times greater than for sodium chloride, it is still acceptable as a reference toxicant. The analytical precision for pentachlorophenol was twice as great as for sodium chloride and may have contributed somewhat to the difference in variability. The coefficients of variation for chromium and Guthion® were considerably higher and varied so much between species that neither of these is suitable as a reference toxicant on the basis of this criterion.

The results of the bioassays can be used only indirectly to assess whether the toxicant would detect abnormal fish by their having a deviant response. If stocks of fish, particularly goldfish, differed during the 2-year period of testing, the experimental design would permit detection of these differences in response to the toxicants by the multiway analysis of variance. Only the sodium chloride bioassays consistently revealed a

Table 13. OCCURRENCE OF SIGNIFICANT DIFFERENCES IN MULTIWAY ANALYSES OF VARIANCE WITH FOUR TOXICANTS[a/]

Toxicant	Species	Source of variance: Stocks of fish	Source of variance: Bioassay apparatus	Source of variance: Slope
		24-hr LC50		
NaCl	Fathead	ns	ns	ns
	Goldfish	ns	ns	ns
PCP	Fathead	ns	ns	ns
	Goldfish	sig	ns	ns
Cr^{+6}	Fathead	ns	ns	ns
	Goldfish	--	--	--
Guthion	Fathead	sig	ns	ns
	Goldfish	--	--	--
		96-hr LC50		
NaCl	Fathead	sig	ns	ns
	Goldfish	sig	ns	ns
PCP	Fathead	ns	ns	ns
	Goldfish	ns	ns	ns
Cr^{+6}	Fathead	sig	ns	ns
	Goldfish	ns	sig	sig
Guthion	Fathead	ns	ns	ns
	Goldfish	ns	ns	ns
		Terminal LC50		
NaCl	Fathead	sig	ns	ns
	Goldfish	sig	ns	ns
PCP	Fathead	ns	ns	ns
	Goldfish	ns	ns	ns

Table 13 (continued). OCCURRENCE OF SIGNIFICANT DIFFERENCES IN MULTIWAY ANALYSES OF VARIANCE WITH FOUR TOXICANTS[a/]

Toxicant	Species	Source of variance: Stocks of fish	Bioassay apparatus	Slope
		Terminal LC50		
Cr^{+6}	Fathead	ns	ns	ns
	Goldfish	ns	ns	ns
Guthion	Fathead	ns	ns	ns
	Goldfish	ns	ns	ns

[a/] "Sig" indicates a significant difference; "ns" indicates no significant difference; per experiment $p<.05$.

significant difference between the eight stocks of fish tested with both the 96-hr and threshold LC50's different for both species (Table 12). Three other indications of significant differences between fish stocks, goldfish with pentachlorophenol at 24 hours and fathead minnows with chromium at 96 hours and Guthion® at 24 hours, were not consistent between species or other test durations. Since the same stock of goldfish was tested with each toxicant, significant differences might be expected with all toxicants. However, bioassay results with sodium chloride were considerably less variable (Table 12) and thus significant differences between stocks tested with the other toxicants were either masked by the greater overall variability or did not occur. Therefore, sodium chloride appears to be the most sensitive indicator of differences between fish stocks. Whether or not these differences are due to previous history of the stock, fish condition at the time of testing, or some uncontrolled variable cannot be evaluated from these data. The mortality of all goldfish stocks during the pre-test holding period was similar (1-10%) so that differences cannot be attributed to this effect.

One attempt was made to determine if a previously unhealthy and stressed

stock of fish could be detected by deviant LC50's when exposed to any of the four toxicants. A stock of goldfish arrived in extremely poor condition, heavily infested with skin flukes and probably a bacterial disease. Daily mortality was high for over a month, and during that period numerous treatments with potassium permanganate, tetracycline, or neomycin were administered. After about 50% of the fish were lost, mortality ceased, and the fish were tested in duplicate with each toxicant. It was hypothesized that these fish would either be more resistant to the toxicants if the weaker individuals had been eliminated from the population, less resistant if the total stress from the diseases and treatments had increased their sensitivity, or equally resistant if the two factors balanced out.

With the unhealthy fish the equivalent 96-hr and threshold LC50's of sodium chloride were considerably lower than the means from all bioassays with the presumed healthy fish and well outside the range of all of these tests. These results correspond to the analyses of variance of the 16 regular bioassays where there were significant differences in 96-hr and threshold LC50's but no difference at 24 hours (Table 13). The reverse trend occurred with pentachlorophenol. Threshold and 96-hr LC50's of the unhealthy fish were within the range of the "normal" fish, but the 24-hr LC50's were greater and outside the range for normal fish (Table 14). These results correspond with the analyses of variance for pentachlorophenol where differences in stocks of goldfish were detected at 24 hours but not at the other two times (Table 13). The only other instances where the LC50's for the unhealthy fish were outside the range for normal fish were the 96-hr and 11-day values for Guthion® (Table 14). The analyses of variance did not detect stock differences with Guthion® at these times, but the great overall variability of Guthion® probably masked any possible differences.

Sodium chloride is most capable of detecting abnormal fish by deviant LC50's but bioassays must be conducted for longer than 24 hours. Pentachlorophenol is very rapid in detecting abnormal fish, but after 24 hours

Table 14. COMPARISON OF LC50'S FROM UNHEALTHY GOLDFISH STOCK WITH MEAN LC50'S FROM ALL TESTS WITH "NORMAL" GOLDFISH (milligrams/liter)

Stock		LC50 NaCl	PCP	Cr^{+6}	Guthion®
		24-hr LC50			
"Normal"	Mean	9952	0.27	-	7.78
	Range	8350-11050	0.18-0.37	-	5.34-11.20
Unhealthy	Test 1	9270	0.38	-	7.35
	Test 2	10270	0.44	-	6.25
		96-hr LC50			
"Normal"	Mean	7341	0.22	120	2.37
	Range	6800-8050	0.17-0.30	90-135	1.35-3.86
Unhealthy	Test 1	6170	0.24	93	< 0.18
	Test 2	6180	0.30	106	0.17
		Terminal LC50[a/]			
"Normal"	Mean	7322	0.21	33	0.80
	Range	6800-8050	0.15-0.28	15-59	0.19-1.22
Unhealthy	Test 1	6170	0.19	37	< 0.18
	Test 2	6180	0.25	56	< 0.17

[a/] Threshold LC50 for NaCl and PCP; 11-day LC50 for Cr^{+6} and Guthion®.

the differences disappear. This difference may give an advantage to pentachlorophenol if reference bioassays are conducted for just 24 hours prior to testing an unknown. Both hexavalent chromium and Guthion® are relatively incapable of discovering abnormal fish.

A reference toxicant should be suitable for use in either flow-through or static systems depending on the needs of the laboratory, but static tests will probably be used more frequently by industries. With the flow-through systems currently in use it is impracitcal to use sodium chloride as a reference toxicant because the quantity of the salt required is too great. Pentachlorophenol can be used for tests in either system. When the same stock of goldfish was tested simultaneously in duplicate static bioassays and duplicate flow-through systems, the threshold LC50's were 1-1/2 to 2 times greater in the static tests. The cause of this difference was not determined. It was not due to deterioration or loss of toxicant in the static system since concentrations were monitored daily and no change occurred. No static bioassays were attempted with hexavalent chromium but others have conducted apparently successful static tests (Trama and Benoit, 1960). Although static Guthion® tests were performed, flow-through tests are possible as was demonstrated in a chronic test conducted in our laboratory. The major difficulty with a flow-through acute bioassay of Guthion® was solution of the high concentrations needed for acute tests.

Table 15 summarizes the ability of the four toxicants to meet the seven desired characteristics of a reference toxicant. The first five criteria (top to bottom) have been discussed previously in depth. Ease of chemical analysis is an important consideration for research laboratories, but industrial laboratories will probably use calculated concentrations without chemical analysis. The subjective judgment on ease of handling is based on use of the chemical in a laboratory including such factors as equipment cleaning and administration of the material and on considerations of degradation of waste material in the environment and toxicity to humans (including carcinogenicity).

Effect of Size and Age

Goldfish--Constant stock--Mean lengths and weights of the three groups of small fish ranged from 34-36 cm and 1.41-1.70 g and for the large fish

Table 15. COMPARISON OF FOUR TOXICANTS WITH REGARD TO THEIR USE AS A REFERENCE TOXICANT[a/]

	NaCl	PCP	Cr^{+6}	Guthion®
Consistency of fish response	1	2	3	4
Detection of abnormal fish	2	2	4	3
Rapid attainment of threshold LC50	1	1	4	4
Use in static bioassay	2	2	2	2
Use in continuous-flow bioassay	4	1	1	3
Ease of chemical analysis	1	3	1	4
General ease in handling	2	2	3	4

[a/] 1 - very good; 2 - good; 3 - fair; 4 - poor.

from 43-44 cm and 2.68-3.59 g (Table 16). The regression equations and the percentage of variability in the reciprocal of LC50 attributable to reciprocal of time of the measurement (coefficient of determination - r^2) are presented in Table 17 for the combined data for groups of small and large fish. An analysis of covariance indicated no significant difference in slope ($F_{1,26} = 1.63$, $p > .05$) or elevation ($F_{1,27} = 3.68$, $p > .05$), thus both large and small goldfish from the same stock responded similarly to the pentachlorophenol (Figure 4). If the 24-hr, 96-hr, and threshold LC50's are compared between the large and small fish (Table 18), the 24- and 96-hr LC50's are not significantly different ($t = 2.49$, $p > .05$ and $t = .390$, $p > .05$) but the threshold LC50's are different ($t = 5.00$, $p = .025$-$.05$). This apparent contradiction of the analysis of covariance results because it compares variability over the entire test period whereas the t-test compares the variability at one instant. Thus, the rate of mortality throughout the entire test was similar for both groups, but the concentration at which mortality ceased (threshold LC50) was greater for the small fish indicating that these were ultimately

Table 16. MEAN AND STANDARD DEVIATION OF WEIGHT AND LENGTH OF FISH IN ALL BIOASSAYS

Test	Age, wks	Nominal size	Weight, g Mean	Weight, g SD	Length, cm[a/] Mean	Length, cm[a/] SD
		Goldfish - Constant Stock				
G1A	-	small	1.41	.336	35	3.2
G1B	-	large	2.68	.652	43	3.0
G2A	-	small	1.62	.455	36	3.0
G2B	-	large	3.15	.751	44	3.4
G3A	-	small	1.70	.426	34	2.5
G3B	-	large	3.59	.838	43	3.1
		Fathead - Constant Age				
F1A	11	small	.09	.037	17	2.4
F1B	11	large	.27	.094	24	2.6
F2A	11	small	.11	.038	16	2.0
F2B	11	large	.28	.094	24	2.6
F3A	11	small	.05	.019	17	1.7
F3B	11	large	.26	.090	29	3.0
		Fathead - Various Ages				
F4A	4	-	.02	.007	13	1.6
F4B	7	-	.04	.025	18	2.0
F4C	11	-	.15	.065	25	3.4
F4D	14	-	.21	.047	28	2.2
F5A	4	-	.03	.014	14	1.9
F5B	7	-	.10	.036	21	2.6
F5C	11	-	.24	.099	27	3.5
F5D	14	-	.29	.123	30	4.1
F6A	4	-	.01	.011	12	2.6
F6B	7	-	.05	.034	17	2.9
F6C	11	-	.16	.072	25	4.0
F6D	14	-	.20	.093	27	4.2

Table 16 (continued). MEAN AND STANDARD DEVIATION OF WEIGHT AND LENGTH OF FISH IN ALL BIOASSAYS

Test	Age, wks	Nominal size	Weight, g Mean	Weight, g SD	Length, cm[a/] Mean	Length, cm[a/] SD
			Fathead - Various Ages			
F7A	4	-	.02	.008	13	1.9
F7B	7	-	.03	.011	15	1.5
F7C	11	-	.21	.090	26	3.6
F7D	14	-	.32	.117	31	3.6

a/Goldfish - standard length; fathead minnow - total length.

slightly less sensitive.

Fathead Minnows—Constant age—Mean lengths and weights of the three groups of small fathead minnows ranged from 16-17 cm and .05-.09 g and for the large fish from 24-29 cm and .26-.28 g (Table 16). The regression equations for the reciprocal toxicity curves with these two groups resulted in the best fit of any groups tested with coefficients of determination of .83 and .88 (Table 17). The slopes of these regression lines were significantly different ($F_{1,33} = 7.59$, $p < .01$) indicating a different rate of mortality over the entire test period (Figure 4). Difference in elevation was not tested since this is meaningless with different slopes. There was no significant difference in 24-hr, 96-hr, or threshold LC50's (Table 18) between the two size groups ($t = 2.00$, $p > .05$, $t = 1.61$, $p > .05$ and $t = 1.61$, p .05, respectively). Although the regression lines were separating at the time of the 24-hr, 96-hr and threshold measurements (Figure 4), differences between the two groups were not sufficient to reveal statistical significance. In summary, the small fathead minnows died sooner at a given concentration but the rate of mortality once death began to occur was greater for the large fish. By the time 24-hr and threshold LC50's occurred there was no difference between sizes.

Table 17. REGRESSION EQUATIONS AND COEFFICIENT OF DETERMINATION (r^2) FOR THE RELATIONSHIP BETWEEN LC50 AND TIME IN THREE GROUPS OF EXPERIMENTS

Experiment	Regression Equation	r^2
	Goldfish	
Small	$1/Y = 4.09 - 11.57\ (1/x)$	.82
Large	$1/Y = 4.82 - 15.05\ (1/x)$	.78
	Fathead - Constant Age	
Small	$1/Y = 4.35 - 8.74\ (1/x)$	.83
Large	$1/Y = 5.05 - 13.28\ (1/x)$	.88
	Fathead - Various Ages	
4 weeks	$1/Y = 4.77 - 11.44\ (1/x)$	.80
7 weeks	$1/Y = 4.09 - 10.15\ (1/x)$	.76
11 weeks	$1/Y = 4.03 - 10.30\ (1/x)$	.71
14 weeks	$1/Y = 4.87 - 14.80\ (1/x)$	.74

Different ages--The mean lengths and weights of fathead minnows increased with age in each experimental group although sizes between different test groups overlapped (Table 16). Density of fish in the culture unit aquaria was probably responsible for this effect. The four regression equations based on concentration against time did not fit the data as well as those related to size of fathead minnows and goldfish, but the coefficients of determination were still adequate (Table 17). Analysis of covariance indicated a significant difference in slopes ($F_{3,150} = 3.16$, $p = .025$-$.05$) so each line was compared with all others by t-tests (Figure 4). The slope of the line for 14-week-old fish was significantly different than for 11-, 7-, and 4-week-old fish ($t = 2.52$, $p < .05$, $t = 2.76$, $p < .05$, and $t = 1.98$, $p = .05$, respectively). None of the other comparisons approached significance.

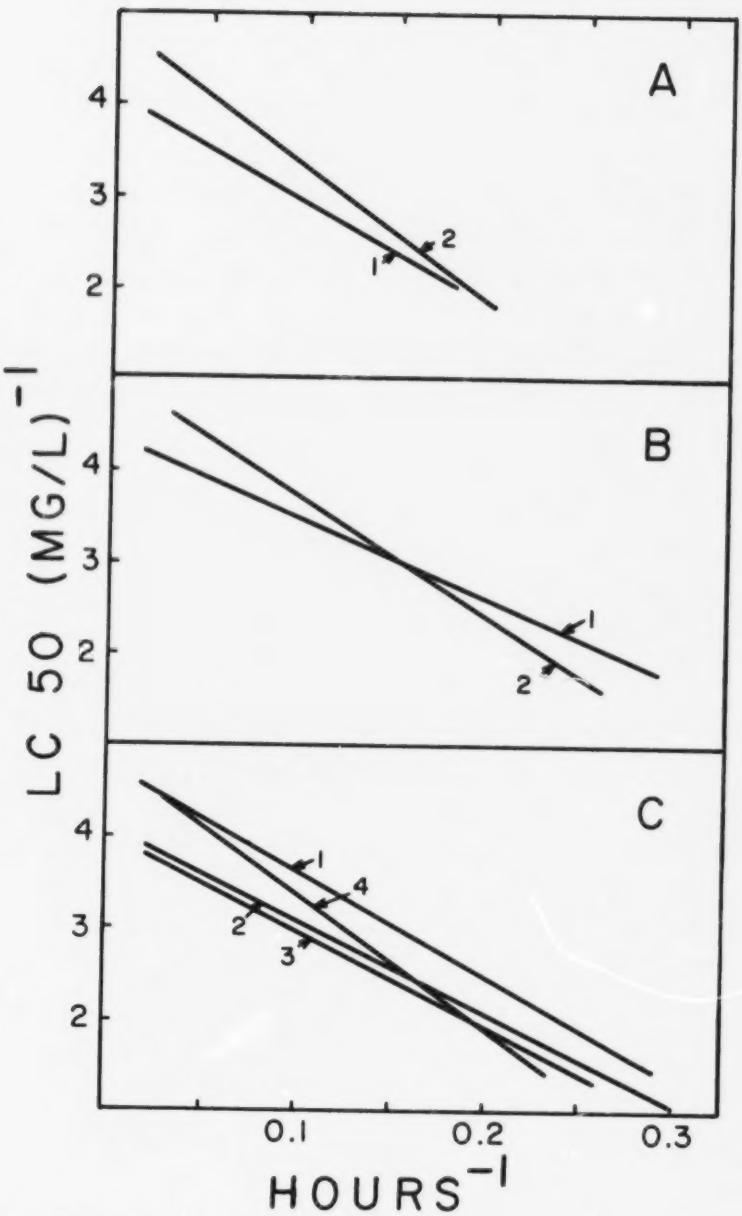

Figure 4. Relationship of reciprocal of LC50 of pentachlorophenol versus reciprocal of time. A: goldfish, 1) small, 2) large; B: fathead minnow - constant age, 1) small, 2) large; C: fathead minnow - different ages, 1) 4 weeks; 2) 7 weeks, 3) 11 weeks, 4) 14 weeks.

Table 18. MEANS AND STANDARD DEVIATIONS OF LC50'S WITH PENTACHLOROPHENOL FOR DIFFERENT GROUPS OF FISH AT VARIOUS TIMES (mg/liter pentachlorophenol)

	24-hr LC50		96-hr LC50		Threshold LC50	
Experiment	Mean	SD	Mean	SD	Mean	SD
			Goldfish - Constant Stock			
Small	.267	.042	.247	.025	.240	.026
Large	.250	.053	.190	.020	.190	.020
			Fathead - Constant Age			
Small	.240	.036	.227	.029	.227	.029
Large	.213	.025	.203	.012	.203	.012
			Fathead - Various Ages			
4 weeks	.222	.021	.198	.017	.198	.017
7 weeks	.245	.039	.230	.036	.230	.036
11 weeks	.232	.052	.222	.039	.222	.039
14 weeks	.200	.016	.190	.012	.190	.012

The 24-hr, 96-hr, and threshold LC50's for the four age groups are presented in Table 18. Analyses of variance indicated no significant differences between different groups of fish. The 4-, 7-, and 11-week-old fish died sooner at a given high concentration but the rate of mortality after death began was greater for the 14-week-old fish than the other three so that at the time of the 24-hr, 96-hr and threshold measurements there was no difference between ages.

GOLDFISH CULTURE STUDY

Test Conditions

The temperature in each tank was maintained close to the desired temperatures with a range in means of 19.85 to 20.02 C for the nominal 20 C tanks

and 24.58 to 25.10 C for the nominal 25 C tanks (Table 19). The ranges in mean pH meter reading overlapped for the 20 and 25 C tanks with 7.48-7.58 for the former and 7.55-7.62 to the latter. Mean total alkalinity was 195.6 (standard deviation 7.2) and 191.3 mg/liter $CaCO_3$ (standard deviation 5.4) for the 20 and 25 C tanks, respectively. Dissolved oxygen was more difficult to maintain in the higher temperature tanks even with the use of air dispersion stones. Mean dissolved oxygen concentrations ranged from 5.29 to 6.36 and from 4.92 to 5.70 mg/liter in the 20 and 25 C tanks, respectively (Table 19). After 115 days an accident occurred which resulted in an increase of the temperature in tank #1 to lethal levels, thereby eliminating this tank from the experiment.

Eggs, Fry and Mortality

The three groups of eggs held at 20 C had survival percentages through hatching of 68.1, 30.2, and 34.1 and the three groups at 25 C survived at percentages of 56.6, 21.0, and 39.0. Total survival was 40% at 20 C and 38% at 25 C indicating no effect of temperature. The relatively low survival was probably due to infertility of the artificially fertilized eggs.

After fry were consolidated within a temperature group no attempt was made to assess mortality until the fish were distributed to individual tanks. During the 29 days from the assignment of 18 fish to each tank until the first thinning to 12 fish per tank, 81% of the fry survived at 20 C and 89% at 25 C. The fish were ultimately thinned to 10 per tank and thereafter occasional fish were lost from accidents or unknown causes. This mortality is reflected in the number of individual fish specified for each tank in Table 20.

Coloration

After 90 days the proportion of fish that had changed from olive-brown to a gold or white coloration was recorded. Analysis of variance after an arcsin transformation of the proportions indicated that temperature

Table 19. MEANS AND STANDARD DEVIATIONS OF CONDITIONS IN GOLDFISH CULTURE STUDY

Nominal temperature, C	Diet	Tank size	Tank number	Temperature, C		pH meter reading		Oxygen, mg/liter	
				Mean	SD	Mean	SD	Mean	SD
20	Straight	Large	2	19.85	1.10	7.55	0.16	5.94	1.10
			7	19.92	1.14	7.57	0.18	5.87	0.94
		Small	4	19.93	1.05	7.54	0.15	5.29	1.17
			8	19.94	1.13	7.58	0.14	5.85	1.04
	Mixed	Large	3	19.98	1.12	7.55	.016	6.36	0.82
			5	20.02	1.02	7.55	0.17	6.31	0.96
		Small	1[a/]	19.87	1.61	7.48	0.13	5.84	0.57
			6	19.95	1.12	7.58	.018	6.26	0.89
25	Straight	Large	10	24.84	1.31	7.56	.014	5.10	0.94
			15	25.10	1.19	7.58	0.14	5.34	0.96
		Small	12	24.65	1.28	7.59	0.14	4.97	1.15
			14	24.77	1.17	7.58	0.13	4.92	1.05
	Mixed	Large	11	24.89	1.22	7.55	0.16	5.27	1.39
			13	24.98	1.17	7.59	0.17	5.70	1.12
		Small	9	24.86	1.21	7.62	0.16	5.38	1.13
			16	24.58	1.15	7.60	0.17	5.01	1.40

[a/] All fish lost after 115 days due to accident.

alone was the only factor showing a significant effect on the rate of color change ($F_{1,8}$ = 54.12, $p<.005$). At 20 C 7% of the fish had completed the color change and 79% had done so at 25 C. Size of the fish versus coloration was not recorded, but direct observation indicated that size was probably not a significant covariate since smaller fish had changed color at 25 C and most larger fish at 20 C had not. Once all fish had turned white or gold, the intensity of the color was much greater in tanks of fish fed the mixed diet, particularly after the fresh duckweed was included.

Growth

Fish in all tanks gained weight throughout the entire test period (Table 21). Analysis of variance indicated significant differences in final weights (Table 20) due to all main factors and one two-factor interrelation. There was a significant difference in weight due to temperature ($F_{1,7}$ = 48.97, $p<.005$), due to food ($F_{1,7}$ = 29.15, $p<.005$), due to tank size ($F_{1,7}$ = 8.29, p = .01-.025), and due to the temperature x food interaction ($F_{1,7}$ = 7.35, p = .025-.05). The temperature x size, food x size, and temperature x food x size interactions were not significantly different ($p>.05$). Weight of the fish was greater at the higher temperature, with the straight Oregon moist diet, and in the larger tanks. The interaction between the 25 C temperature and the straight Oregon moist diet also resulted in greater weight gain.

Analysis of variance of final lengths (Table 22) also indicated significant differences due to temperature ($F_{1,7}$ = 63.56, $p<.005$), due to food ($F_{1,7}$ = 6.01, p = .025-.05), and due to tank size ($F_{1,7}$ = 14.52, p = .005-.01). None of the two-factor interactions resulted in significant differences in final lengths ($p>.05$), but the three-factor interaction of temperature, food, and tank size did result in a significantly greater length ($F_{1,7}$ = 7.51, p - .025-.05) with higher temperature, straight Oregon moist diet, and larger tank size.

Table 20. WEIGHT OF INDIVIDUAL FISH IN ALL TANKS AT TERMINATION AFTER 393 DAYS

(grams)

Replicate tank	Fish number[a/]	Nominal 20 C				Nominal 25 C			
		Straight diet		Mixed diet		Straight diet		Mixed diet	
		Large tank	Small tank	Large tank	Small tank	Large tank	Small tank	Large tank	Small tank
1	1	22.0	38.5	25.0	-[b/]	49.0	35.5	18.5	20.0
	2	55.0	41.0	42.0	-	50.0	47.0	59.0	45.5
	3	74.0	53.5	57.0	-	50.5	59.5	75.0	59.5
	4	75.5	55.5	73.5	-	57.0	78.5	91.0	66.0
	5	79.0	71.0	77.5	-	80.5	124.0	95.5	67.5
	6	83.0	83.5	82.0	-	110.0	135.5	98.0	78.0
	7	86.0	90.5	82.5	-	111.5	146.5	102.5	88.5
	8	92.0	103.0	94.5	-	197.0	148.0	135.0	92.5
	9	105.5	121.5	97.0	-	237.0	179.5	143.0	151.5
	10	185.0	147.5	104.5	-	274.5	-	149.5	199.5
	Mean	85.70	80.55	73.55		121.70	106.00	96.70	80.85
2	1	24.5	55.5	61.0	14.0	75.5	23.0	25.0	34.5
	2	58.0	60.0	66.0	31.5	89.5	26.5	55.5	43.5
	3	65.5	63.0	73.5	55.0	94.5	98.5	64.5	49.5
	4	87.5	71.0	80.0	55.0	124.0	119.5	69.0	56.0
	5	97.5	78.5	100.5	75.0	136.0	120.0	71.5	57.0

Table 20 (continued). WEIGHT OF INDIVIDUAL FISH IN ALL TANKS AT TERMINATION AFTER 393 DAYS
(grams)

Replicate tank	Fish number[a]	Nominal 20 C				Nominal 25 C			
		Straight diet		Mixed diet		Straight diet		Mixed diet	
		Large tank	Small tank	Large tank	Small tank	Large tank	Small tank	Large tank	Small tank
2	7	102.5	85.0	110.5	107.5	155.5	133.5	81.0	128.5
	8	104.0	92.5	113.5	154.5	156.5	171.5	120.0	130.5
	9	141.0	119.0	122.0	-	186.5	202.5	161.0	152.0
	10	151.0	141.0	-	-	-	-	162.0	-
	Mean	93.05	84.40	92.00	71.75	128.27	112.83	89.05	91.70

[a] Number corresponds to fish listed in Tables 22 and 23.
[b] Fish lost after 115 days due to accident.

Table 21. MEAN WEIGHTS OF FISH FROM ALL INDIVIDUAL TANKS AT APPROXIMATELY MONTHLY INTERVALS (grams)

Days after start	Replicate tank	Nominal 20 C				Nominal 25 C			
		Straight diet		Mixed diet		Straight diet		Mixed diet	
		Large tank	Small tank	Large tank	Small tank	Large tank	Small tank	Large tank	Small tank
70	1	1.92	1.44	2.10	0.98	4.67	4.09	3.17	2.91
	2	2.47	1.67	2.56	1.33	4.04	3.59	3.21	3.21
99	1	6.46	6.14	6.37	4.63	10.28	10.74	8.43	7.92
	2	8.19	7.40	7.48	5.46	10.08	10.22	7.14	7.59
127	1	12.73	13.59	12.29	10.50	16.12	17.13	13.69	12.66
	2	15.33	14.78	13.34	10.45	15.72	16.67	11.72	13.26
158	1	21.15	22.98	19.05	- [a/]	25.67	25.31	20.37	19.23
	2	25.16	23.48	20.75	17.13	24.69	25.02	19.60	20.41
188	1	32.01	30.92	26.85	-	36.38	34.64	29.00	27.11
	2	34.57	31.75	28.43	24.31	35.54	33.19	25.26	28.87
219	1	43.99	42.41	35.43	-	55.19	48.49	38.26	37.00
	2	49.00	43.02	38.69	32.21	48.49	47.55	35.77	40.18
250	1	56.64	53.70	46.77	-	71.76	63.10	52.19	51.00
	2	63.08	56.58	57.57	43.16	75.46	62.46	50.42	55.62
280	1	67.46	63.63	57.36	-	88.03	74.22	70.44	63.02
	2	75.35	67.53	70.57	52.84	89.43	79.02	61.83	58.42

Table 21 (continued) MEAN WEIGHTS OF FISH FROM ALL INDIVIDUAL TANKS AT APPROXIMATELY MONTHLY INTERVALS

(grams)

Days after start	Replicate tank	Nominal 20 C				Nominal 25 C			
		Straight diet		Mixed diet		Straight diet		Mixed diet	
		Large tank	Small tank	Large tank	Small tank	Large tank	Small tank	Large tank	Small tank
310	1	74.67	71.89	62.87	-	98.23	86.21	79.70	70.15
	2	81.00	74.35	78.72	58.11	100.32	88.67	71.81	65.46
340	1	82.51	78.01	72.14	-	112.67	93.72	89.62	80.62
	2	90.07	79.09	87.56	66.27	114.67	97.96	81.48	82.46
393	1	85.70	80.55	73.55	-	121.70	106.00	96.70	86.85
	2	93.05	84.40	92.00	71.75	128.27	112.83	89.05	91.70

a/ Fish lost after 115 days due to accident.

Table 22. STANDARD LENGTHS OF INDIVIDUAL FISH IN ALL TANKS AT TERMINATION AFTER 393 DAYS (millimeters)

Replicate tank	Fish number[a]	Nominal 20 C				Nominal 25 C			
		Straight diet		Mixed diet		Straight diet		Mixed diet	
		Large tank	Small tank	arge tank	Small tank	Large tank	Small tank	Large tank	Small tank
1	1	76	83	75	-[b]	99	89	74	80
	2	96	92	94	-	105	99	110	100
	3	114	96	99	-	94	101	111	107
	4	124	104	119	-	108	121	128	115
	5	117	107	120	-	125	143	126	120
	6	121	112	121	-	128	139	132	115
	7	119	113	123	-	139	150	139	136
	8	117	117	128	-	166	139	150	137
	9	128	132	128	-	177	160	150	160
	10	160	142	134	-	189	-	153	177
	Mean	117.2	109.8	114.1	-	133.0	126.8	127.3	124.7
2	1	74	99	102	64	111	77	87	79
	2	102	99	107	84	119	85	106	97
	3	92	112	120	84	124	125	113	105
	4	112	110	120	94	135	137	113	107
	5	125	100	134	112	142	136	115	139
	6	122	118	132	116	143	125	115	118
	7	125	123	132	130	153	144	125	139
	8	127	128	135	145	146	147	142	148
	9	136	133	140	-	151	159	156	153
	10	146	141	-	-	-	-	156	163
	Mean	116.1	116.3	124.7	103.6	136.0	126.1	122.8	124.8

[a] Number corresponds to fish listed in Tables 20 and 23.
[b] Fish lost after 115 days due to accident.

Maturation

At termination of the experiment the gonads were examined and a subjective judgment on the degree of maturity was made using the following classification (Table 23):

I. Immature - gonads extremely small and difficult to locate with the naked eye

II. Resting stage - sexual products have not yet begun to develop; gonads very small but easily located

III. Maturation - gonads of moderate size; eggs not distinguishable to the naked eye and milt not running when pressure is applied to abdomen

IV. Maturity - gonads have nearly achieved maximum size; eggs distinguishable to naked eye, but not extruded with pressure; milt is extruded under light pressure and some secondary sexual characteristics are visible.

Although fish were large enough and old enough to spawn based on our previous experience with field-reared fish, only one female and two males had mature gonads. These three fish all were in a large, 25-C tank and fed the mixed diet, but the replicate of this treatment was similar to all other tanks (Table 23). The presence of the three most mature fish in the same tank may indicate an uncontrolled and unknown variable influencing those fish.

To determine if any experimental condition resulted in more mature fish, an analysis of variance was calculated on the percentage of III and IV group fish in each tank after an arcsin transformation of the percentages. No significant differences were detected between treatments, factors, or factor interactions. This experiment was unsuccessful in providing conditions suitable for complete maturation within a desired 1-yr period.

Table 23. SEX AND STATE OF GONADAL MATURITY[a/] OF INDIVIDUAL FISH IN ALL TANKS AT TERMINATION AFTER 393 DAYS

Replicate tank	Fish number[b/]	Nominal 20 C				Nominal 25 C			
		Straight diet		Mixed diet		Straight diet		Mixed diet	
		Large tank	Small tank	Large tank	Small tank	Large tank	Small tank	Large tank	Small tank
1	1	F-II	F-II	F-II	- [c/]	M-II	M-II	F-II	M-II
	2	F-II	F-II	M-II	-	F-II	M-II	M-III	F-II
	3	F-II	M-I	F-II	-	M-II	F-II	M-III	M-I
	4	F-II	F-II	M-III	-	M-III	F-II	F-II	M-I
	5	M-III	M-III	F-II	-	M-II	F-II	M-IV	F-II
	6	F-I	M-III	M-II	-	F-II	M-II	M-IV	M-II
	7	M-II	M-III	M-II	-	F-II	M-II	F-II	F-II
	8	F-II	M-II	M-II	-	M-III	F-II	F-II	F-II
	9	F-II	F-II	F-II	-	F-II	F-II	M-III	F-II
	10	F-II	F-II	M-II	-	M-II	-	F-IV	M-II
Female (%)		80	50	40	-	40	55.6	50	50
III & IV (%)		10	30	10	-	20	0	60	0
2	1	M-I	M-I	M-I	M-I	M-II	F-II	F-II	M-II
	2	M-II	F-II	F-II	F-II	F-II	M-II	F-III	M-II
	3	M-II	M-III	M-I	F-II	F-II	M-II	M-II	M-II
	4	F-II	M-II	F-II	F-II	F-II	M-II	F-II	F-II
	5	F-II	M-II	F-II	M-II	M-III	M-II	F-II	M-III
	6	F-II	M-III	F-II	M-II	M-II	M-II	F-I	M-II
	7	F-II	F-II	F-II	F-II	M-II	M-II	M-II	M-II
	8	F-II	F-II	F-II	F-II	F-II	M-II	M-II	F-II
	9	M-II	F-II	F-II	-	M-II	M-III	F-II	F-II
	10	M-II	F-II	-	-	-	-	M-III	M-II
Female (%)		50	50	77.8	62.5	44.4	11.1	60	30
III & IV (%)		0	20	0	0	11.1	11.1	20	10

[a/] Roman numeral maturity rating discussed in text.
[b/] Number corresponds to fish listed in Tables 20 and 22.
[c/] All fish lost after 115 days due to accident.

SECTION VI

DISCUSSION

SELECTION OF A STANDARD SPECIES

Acute Toxicity

A desirable attribute of a standard fish species would be moderate sensitivity to many toxicants. Fish with extreme sensitivity would require tremendous dilution of effluents for proper concentrations in an acute test and chemical analysis of low concentrations might be less accurate or precise. Extreme resistance to many toxicants might require such high concentrations of industrial effluent that no kill could be attained with full strength effluents. Either situation would add complexities to standard bioassays meant to be conducted with only semi-experienced technicians. Since little data in the literature on the toxicity of sodium chloride, pentachlorophenol, hexavalent chromium, or Guthion® is comparable to the present study because different bioassay conditions were maintained, the sensitivity of the fathead minnow and goldfish cannot be compared to other species, only to each other.

The toxicity curves indicate that although mortality of fathead minnows from three of the toxicants (pentachlorophenol, sodium chloride, and Guthion®) is initially more rapid than mortality of goldfish, by termination of the tests goldfish were at least as sensitive, if not more so, than the fathead minnows. The toxicity curves for hexavalent chromium are incomplete but goldfish may ultimately also be more sensitive to this toxicant (Figure 2). The initial resistance of the goldfish make that species less desirable as a standard since some industries

may use 24-hour tests.

Variability of Bioassay Results

On the basis of minimum variability in results of acute bioassays, neither the goldfish nor the fathead minnow was superior, and variability was more dependent on the toxicant than on the species. With the toxicants for which a threshold LC50 was attained rapidly, sodium chloride and pentachlorophenol, variability was relatively small, but with Guthion® and hexavalent chromium, where a threshold LC50 was not attained by 11 days, variability was considerably greater (Table 12). Thus the decision on selection of a standard fish between these two species must be based on other criteria.

Goldfish Culture and Other Criteria

Both species are easily handled for bioassay purposes; however smaller test containers can be used for fathead minnows since even 14-week-old fathead minnows are considerably smaller than the size of goldfish available from Ozark Fisheries. Both species should be easily transportable since both are tolerant of low oxygen concentration. The fathead minnow has been used successfully in many chronic studies since sexual maturity can be achieved in about 3 to 5 months. The goldfish culture study was unsuccessful in the attempt to raise fish from an egg to spawning adult in one year, and we are not aware of anyone having done so under laboratory conditions. Further studies are necessary to achieve success. On the basis of these biological considerations, the fathead minnow is recommended as the better of the two species for a standard bioassay fish. Since the goldfish is available from a large commercial source it could be used at present, although stocks obtained during the summer are in poor condition and generally cannot be used for bioassay. Regulating agencies could probably promote the development of a source for fathead minnows. Fathead minnows from a variety of sources may be undesirable if they vary considerably in resistance to a toxicant.

Size and Age of Standard Fish

Since the threshold LC50's for the large and small goldfish exposed to pentachlorophenol were significantly different (Table 18), it may be desirable to restrict the size selected for bioassay when that species is used as a standard. From a practical point of view, however, the actual difference in LC50's was so small that this size restriction is probably unnecessary. Variability resulting from factors other than size was just as large (Table 3).

Although different rates of mortality occurred between large and small fathead minnows, this difference affected LC50's only at very early time intervals (Table 18). Most standard bioassays require a minimum of 24 hours and LC50's computed at or subsequent to that time were not significantly different. Therefore, size selection of constant age fathead minnows would be unnecessary for pentachlorophenol.

A similar result occurred with fathead minnows of different ages. Although rates of mortality differed, there was no significant difference between LC50's computed at 24 hours or longer with 4-, 7-, 11-, and 14-week-old fish (Table 18). If culture units similar to that described by the U.S. EPA (1971) are used for rearing fathead minnows, various age groups could be mixed to increase numbers available for bioassay. However, since fish of various ages were only tested with pentachlorophenol we cannot be sure if differences due to age might occur with other toxicants. It is therefore recommended that only the 4- or 7-week-old fish be tested. By using younger fish, culture unit production could be increased. These smaller fish would also allow use of smaller bioassay chambers or more fish per chamber. The main disadvantage of using the small fish is difficulty of observation in a large chamber, but this criticism is minor provided observations are made carefully.

SELECTION OF REFERENCE TOXICANT

On the basis of minimum variability and limited information on the

ability to detect abnormal fish, of the four toxicants tested sodium chloride best met the requirements for a reference toxicant (Table 15). Although pentachlorophenol can be used and may have the advantage of more rapid detection of abnormal fish, careful consideration must be given to pH of the test water since toxicity is greatly influenced by pH (Crandall and Goodnight, 1959). This necessity might complicate industrial bioassays. Additional studies are needed to determine clearly the ability of both sodium chloride and pentachlorophenol to detect abnormal fish.

Use of any reference toxicant will require previous standardization by many bioassays to establish a baseline with which comparisons can be made. In establishment of this baseline, complete information on the fish stocks will be required to eliminate stressed fish from consideration. Since even presumed healthy fish vary, rejection of a bioassay stock should be considered only if it falls 1 1/2 to 2 standard deviations outside of the mean of baseline tests. Anything more restrictive would result in rejection of many stocks of relatively normal fish. Stocks falling outside of this criterion would be expected to be suffering from some known or unknown abnormality.

SECTION VII

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SECTION VIII

PUBLICATIONS

Adelman, I. R., L. L. Smith, Jr., and G. D. Siesennop. Acute Toxicity of Sodium Chloride, Pentachlorophenol, Guthion®, and Hexavalent Chromium to Fathead Minnows (Pimephales promelas) and Goldfish (Carassius auratus). J. Fish. Res. Board Can. 33:000, 1976.

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SECTION IX

GLOSSARY

Bioassay - A toxicity test: the estimation of the strength of a poison by its effect on a living organism.

Bioassay, acute - A toxicity test of short duration, usually less than 1 weeks.

Bioassay, chronic - A toxicity test of long duration, usually 1 month or longer.

Bioassay, flow-through - A toxicity test where the toxicant and diluent water are continuously replaced by fresh material.

Bioassay, renewal - A toxicity test where the entire toxicant and diluent water are replaced at period intervals; in this study every 2 or 3 days.

Coefficient of Determination (r^2) - The proportion of the sum of squares of the dependent variable that can be attributed to the independent variable.

Coefficient of Variation (CV) - The sample standard deviation expressed as a percentage of the mean.

Error rate, per comparison - The probability of occurrence of one or more false significant differences among comparisons of different treatments.

Error rate, per experiment - The probability of occurrence of one or more false significant differences among comparisons from all experiments.

Fry - Postembryonic fish up to about 10 days old.

Guthion (azinphos-methyl) - An organic phosphorus insecticide - chemical name: O,O-Dimethyl S-[4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl] phosphorodithioate.

Homeostasis - A state of physiological equilibrium, in this case where processes of excretion or detoxification just balance the toxic action of the poison.

Juvenile - Fish older than 10 days but not sexually mature.

LC50 - The concentration of poison that will kill 50% of the test organisms at a specified time.

LC50, threshold - The concentration of poison at which the organism presumably reaches homeostasis with the toxicant; arbitrarily indicated by 2 days of no deaths in the bioassay.

Type I Error - A statistical error where the experimenter rejects the null hypothesis and it is true.

Toxicity Curve - A mathematical description of the effect of a toxicant where the LC50 is the independent variable, plotted on the ordinate, and time the dependent variable, plotted on the abscissa.

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